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CONTENTS

Comparison of ascospores and conidia of Pyrenophora bromi for incitement of brown leaf spot of Bromus inermis — M. J. Kaufmann, P. N. Drolsom, and E. L. Nielsen 587

The effect of postinoculation temperature on seedling reaction of selected wheat varieties to stem rust — K. R. Bromfield 590

Movement of nonvolatile, diffusible fungicides through columns of soil — Donald E. Munnecke 593

Comparative pathogenicity of Sclerotium rolfsii and Rhizoctonia solani to Spanish peanut — L. J. Ashworth, jr., B. C. Langley, and W. H. Thames, jr. 600

Economic significance of beet Western yellows (radish yellows) on sugar beet — James E. Duffus 605

Relation of some edaphic factors to Agrobacterium tumefaciens — Robert S. Dickey 607

Relative concentration of cucumber mosaic virus in a resistant and a susceptible cucumber variety — Slearm L. Wasuwat and J. C. Walker 614

Effects of concentration of host nutrients on parasitism of Piptocephalis xenophila and P. virginiana — A. L. Shigo, C. D. Anderson, and H. L. Barnett 616

Germination of chlamydospores of Fusarium solani f. phaseoli in unsterilized soils — T. A. Toussoun and William C. Snyder 620

Effects of storage environment on "in vitro" hatching of larvae from cysts of Heterodera schachtii Schmidt, 1871 — D. R. Viglierchio 623

Colonization of organic matter in the soil by fungi — L. E. Tyner 625

Phytophthora megasperma, the principal cause of seed-piece rot of sugarcane in Louisiana — T. van der Zwet and I. L. Forbes 634

Phytopathological Note

Fungi associated with larvae of Sitona spp. — R. A. Kilpatrick 640

Report and abstracts of the 37th annual meeting of the Southern Division of The American Phytopathological Society 642

Bibliography of reviews, 1960 647

News 662

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COMPARISON OF ASCOSPORES AND CONIDIA OF PYRENOPHORA BROMI FOR INCITEMENT OF BROWN LEAF SPOT OF BROMUS INERMIS

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SUMMARY

Smooth bromegrass clones were inoculated with *Pyrenophora bromi* to develop a reliable method of inciting disease for greenhouse screening of breeding material. Ascosporic and conidial inoculations were compared. Disease development incited by ascospores was superior to that from conidia.

INTRODUCTION.—The development of strains of smooth bromegrass (*Bromus inermis* Leyss.) with a high degree of resistance to *Pyrenophora bromi* (Died.) Drechsl. has been retarded because of the lack of a suitable and reliable technique for inciting artificial epiphytotics. Although this disease has not been a major deterrent to the use of smooth bromegrass for forage in the upper Midwestern states, the severity of infection in breeding nurseries during some years is indicative of the potential damage if widespread infection occurred. Severe infection has not been observed where the grass is grown in association with alfalfa, and grazed. Considerable damage, however, has occurred in spring growth cut for hay, silage, or green-feeding, particularly during excessively moist seasons. In some fields most leaves below heights of 12-15 in. have been killed by this fungus. The aftermath usually is not affected by brown leaf spot. Damage to aftermath, however, in some Wisconsin hay meadows, was considerable during the summers of 1958-59.

This study examined several techniques employed by other workers and the relative effectiveness of ascospores and conidia in inciting brown leaf spot of smooth bromegrass (5).

LITERATURE REVIEW.—Increasing the level of resistance to brown leaf spot has been hindered by scanty and spasmodic production of conidia in culture by isolates of the fungus in which considerable genetic variability exists (1, 3, 4, 6). Inoculation of bromegrass with mycelial-conidial suspensions, by the authors, has generally produced unsatisfactory artificial epiphytotics of the disease. The lesions have been variable in number, small, and atypical. The lesions often do not develop the halo characteristic of the disease as it occurs on the host in the field.

Chamberlain and Allison (3) inoculated plants with ascospores produced on potato-dextrose agar (PDA). Disease development was best at 16-20°C. and differences were found in reaction among inbred lines of *B. inermis*. Carnahan and Graham (1) used a mixture of ascospores produced on a grain culture, and conidia with mycelia grown on agar, in greenhouse inoculations. Nineteen *Bromus* spp. were evaluated. There was a high degree of resistance to brown leaf spot only in certain weedy annual species. Emery and Dunn (4) reported that significantly different reactions occurred among bromegrass strains and varieties in their reactions to *P. bromi*. Strains responded similarly during successive years. Significant correlations for reaction to *P. bromi* were noted among clones and their open-pollinated progeny inoculated with conidia or ascospores. They reported sparse conidial formation on agar cultures from which unsatisfactory infection resulted. Differences in severity of infection were associated with the relative numbers of mature ascospores in a given culture.

Tamimi and Dunn (6) inoculated bromegrass with conidia produced on agar and with ascospores produced on grain. Extremely poor conidial production was obtained. Ascospores were much more effective in inciting the disease.

Carter and Dickson (2) noted differences in the amount of disease among resistant or susceptible lines, inoculated with conidia. Disease development in the greenhouse was most severe at 24-28°C.

MATERIALS AND METHODS.—Isolates of *P. bromi* were obtained from infected bromegrass leaves, collected in several Wisconsin localities. Infected tissue was plated on potato-dextrose or water agar following surface sterilization for 60 seconds in 70% alcohol. Solutions of 1.5% sodium hypochlorite or 0.001% mercuric chloride were equally effective sterilants. Tissue was rinsed in tap water after sterilization.

Six of the 60 isolates cultured produced at least a moderate number of conidia on PDA. The 6 isolates were increased on soybean meal or V-8 juice agar.

Ascospore production.—The grain medium used for the production of ascospores consisted of equal parts by volume of oats and barley, boiled in water for 1 hour, after which 500-ml or 1-liter flasks were filled to two-thirds volume with the grain mixture and autoclaved 2 hours at 15-lb pressure.

Many of the 60 isolates produced numerous perithecial primordia within 2 weeks after seeding. Ten of these were increased on PDA or on a barley-oat medium for ascospore production. The 3-4-week-old cultures were stored at 6°C for another 4 months for

production of mature ascospores. It was necessary to select cultures microscopically, since some, especially those grown on the grain medium, did not produce ascospores. Ascospores from 3 or 4 isolates were used in each trial. Those used were not necessarily the same for the different inoculations.

Random isolates that produced numerous perithecia on agar were used to seed either the PDA or the grain medium for the production of ascospores. Mass transfers were employed throughout.

Six to 8 such random cultures grown on PDA in petri dishes or 300-350 ml of the grain medium were mixed with 1 liter of tap water. This was agitated in a blender for 2.5-3 minutes. Cultures grown on PDA were not strained after agitation. Those grown on the grain medium were passed through a tea strainer to remove the larger extraneous particles. The suspensions were sprayed on potted plants at a rate of approximately 1 liter of the mixture to every 240 potted plants.

Conidial production.—Cultures on soybean meal agar were maintained, during 1958-59, at a 16°C night temperature alternating with the laboratory daytime temperatures. Two hundred g of whole soybean meal were boiled in 1 liter of distilled water for 45 minutes, and subsequently strained through 1 thickness of cheesecloth. Twenty g each of Cerelose and agar were added, and the volume of the filtrate restored to 1 liter by the addition of distilled water before sterilization at 15 lb for 30 minutes. Approximately 3 weeks after seeding in petri dishes or prescription bottles, the cultures contained moderate numbers of conidia. In 1959-1960, V-8 juice agar, prepared in the usual manner, was found to be much more satisfactory for conidial production. Cultures were incubated at 20-22°C. Three weeks after seeding most cultures sporulated abundantly.

Six to 8 conidial cultures, at least 1 from each of the 6 better sporulating isolates, together with the medium, were agitated 60-90 seconds in a blender containing 1 liter of tap water. About 1 liter of this mixture was sprayed on every 240 potted plants.

Pretreatment, inoculation, and scoring.—Plants were pretreated in a moist chamber for at least 4 hours. Following inoculation, they were kept in the moist chamber 48-96 hours prior to placement on benches in a greenhouse. The greenhouse was kept at 18-24°C at a relative humidity of at least 50% and usually higher (up to 90% at night). Disease symptoms appeared within 4 days after inoculation, and plants were scored 10-14 days following inoculation.

Clones of smooth bromegrass were grown in 4-in. earthenware pots. Inoculations were made when vegetative growth was 6-10 in. tall.

Twenty-six clones, representing a wide range of morphological plant types and sources, were included in trial 1, conducted during March, 1959. Three replications were inoculated with either ascospores or conidia. All plants selected for study had been scored previously for reaction to infection by *P. bromi* in the field. The plants selected represented a variety of reaction types.

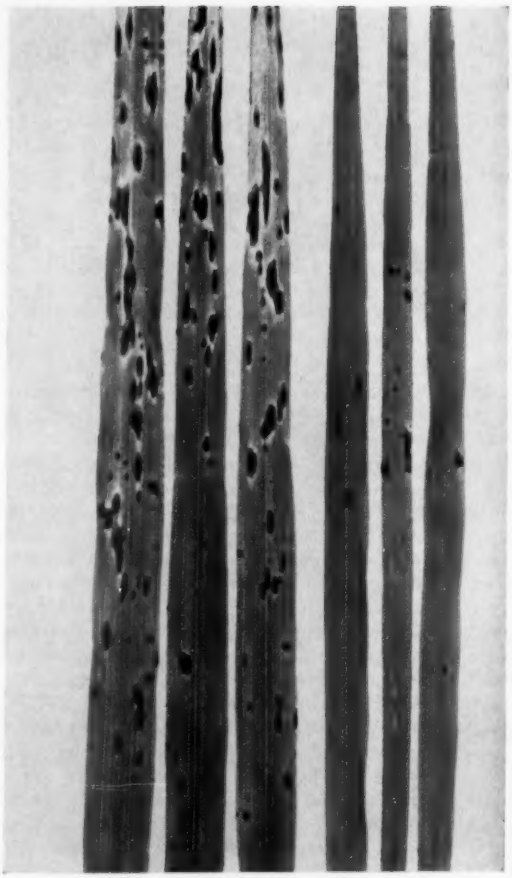

Fig. 1. Leaves from 1 plant of *Bromus inermis* inoculated with ascospores (left) and conidia (right) of *Pyrenophora bromi.*

Sixty-four clones, including 6 of those tested in 1959, were included in the 1960 experiments. Aside from the exception indicated, the experiments were identical.

Following the scoring of plants in trials 2 and 3, all vegetative growth was removed leaving a 1-in. stubble. The plants then were fertilized with 10-10-10 commercial fertilizer and allowed to recover. The plants of the replications inoculated with ascospores in trial 2 were inoculated with conidia in trial 3, and again with ascospores in trial 4. Similarly, treatments with conidia were alternated with those employing ascospores.

Certain bromegrass clones, damaged materially by ascospore inoculations, were eliminated in subsequent trials. Their absence is reflected in the reduced number of plants tested in trials 3 and 4.

TABLE 1. Number of *Bromus inermis* clones in each lesion-type and coverage class following inoculation with ascospores and conidia of *Pyrenophora bromi*

Trial[a]	Inoculum[b]	Lesion type[c]						Coverage class[c]					
		0	1	2	3	4	5	0	1	2	3	4	5
1	A	0	0	1	5	2	18						
	C	3	8	6	8	0	1						
2	A	2	0	1	13	19	29	2	4	7	13	28	9
	C	2	21	10	26	5	0	2	15	13	17	10	6
3	A	1	0	1	7	28	25	1	0	1	9	19	32
	C	3	42	14	3	0	0	6	28	27	1	0	0
4	A	0	0	1	4	11	41	0	0	3	7	22	25
	C	2	21	12	17	2	1	5	23	11	17	2	1

[a] Trials read 3-29-59; 1-29, 4-1, and 4-27-60, respectively.
[b] A: ascospores; C: conidia.
[c] Average of 3 replications.

Results of the artificial inoculations were evaluated by 2 classifications by 2 different workers. Lesion types were scored 0-5; with 0 indicating no infection; 1, a minute spot or fleck-type infection; and 5, large, well-developed lesions surrounded by conspicuous halos. Coverage was classified also on a basis of 0-5. Zero indicated no infection; 1, a few small, scattered lesions; and 5, complete or nearly complete leaf coverage. Scores of 2-4 indicated intermediate lesion types or coverage.

EXPERIMENTAL RESULTS.—The relative effectiveness of the 2 kinds of spores used to incite infection is clearly indicated in the results (Table 1, Fig. 1). Inoculation with ascospores resulted in the development of large lesions and excellent coverage. Estimates of coverage were not recorded in trial 1. Comparatively few plants escaped infection. Lesions that developed from infection incited by conidia were usually smaller, often only tiny spots, than ascospore lesions. Large or susceptible-type lesions occasionally developed from conidia, but they were the predominant type from ascospores. The data relating to the amount of coverage constitute essentially a repetition of the lesion-type data. Coverage after conidial inoculations was inferior to that after ascospore inoculations. Agreement between replications and trials was consistently good and sufficiently definitive without statistical analysis.

DISCUSSION.—Two different populations of bromegrass clones were employed in these investigations, and consistent results were obtained with ascospore inoculation. Therefore, it can be assumed that the use of ascospores to incite infection would be most effective for the evaluation of breeding stocks. The results suggest that conidia may be of little importance in bringing about heavy infections of the organism under greenhouse conditions. It has not been determined if this is true under field conditions.

The arrested lesion development encountered when relative humidity was low in some greenhouse trials indicates the need for relatively high humidity for lesion enlargement. The cyclic development of the disease suggests that low humidity may play an important role in reduced field infection. In the upper Midwest, secondary infection following the spring growth usually is minimal. Extensive development of brown leaf spot on other than the spring and early summer growth has usually occurred during periods of excessively high precipitation and prolonged high relative humidity. Further understanding of this phase of the disease, however, requires exploration.

The object of the studies was to establish a method for reliably inciting induced epiphytotics of brown leaf spot. Although the number of trials was limited, the wide range of genotypes tested and the consistent results obtained suggest that this objective may have been attained adequately. As yet, no attempt has been made to employ this technique for the evaluation of breeding stocks. Perhaps, however, certain precautions are necessary so that artificial inoculation with ascospores can be effective for screening. Perithecia should be examined for the presence of abundant mature ascospores prior to maceration of the culture for the release of spores. Nonsporulating cultures can be retained for subsequent spore production. Some may have to be discarded because of failure to produce spores. If ascospores form in the cultures, the suspension obtained after maceration and straining should be examined microscopically to determine the concentration of spores necessary to incite differential infection on test plants. Calibration of the number of spores applied to a given unit area appears necessary for reasonable uniformity during screening operations. Results may be variable without these precautions. Disregard of the precautions might result in 1) lack of infection through the employment of immature or nonfruiting cultures or 2) development of infections severe enough to mask differences in resistance among clones. The latter is well illustrated in trials 2-4, inclusive.

It appeared immaterial whether PDA or grain cultures were used provided that mass transfers of a number of cultures were employed. This statement is predicated on the fact that this study was not based upon single-spore cultures. The ease of handling and the greater reliability of ascospore production on PDA suggest its superiority to a grain medium for ascospore production. Cultures require at least 4 months of cold treatment for ascospore maturation. Beyond maturation, ascospores have maintained their viability and pathogenicity for at least a year. Thus, good sporulating cultures can be stored.

Although ascospores were superior to conidia for disease incitement, the limited genetic variability of the pathogen sampled and the ecological factors tested do not justify the exclusion of conidial infections.

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THE EFFECT OF POSTINOCULATION TEMPERATURE ON SEEDLING REACTION OF SELECTED WHEAT VARIETIES TO STEM RUST

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SUMMARY

The rust reactions of seedlings of 87 varieties of wheat to *Puccinia graminis* var. *tritici* races 17, 38, and 56 were determined at greenhouse temperatures of 70° and 85°F. With respect to a given race, the varieties were: 1) resistant at both temperatures, 2) susceptible at both temperatures, or 3) resistant at 70° but susceptible at 85°F.

Certain varieties that became susceptible at high temperature (temperature-sensitive varieties) were further tested at 70°, 77°, and 85°F. Twenty varieties inoculated with race 17, 12 inoculated with race 38, and 15 inoculated with race 56 were resistant at 70°F but susceptible at 77° and 85°F.

The reactions of 7 temperature-sensitive varieties to races 11, 15B, 17, 38, and 56 were observed within the critical temperature zone in which breakdown in rust resistance occurs (70-77°F). Individual differences in behavior were noted among the various host-race combinations but, in general, the varieties were: 1) resistant at 70°F, 2) mixed in reaction at 72-74°F, and 3) susceptible at 76°F.

INTRODUCTION.—It is well known (1, 2, 3, 4, 7, 8) that temperature may greatly alter the reaction of certain varieties and selections of wheat to certain races of stem rust [*Puccinia graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Shear, Ball, Jacks. & Stakman]. Temperature modification of reaction introduces complications in the testing of varieties for rust resistance and in the identification of physiologic races and biotypes of rust fungi. It is also of basic scientific interest in connection with the physiology of host resistance, rust physiology, and host-parasite interactions.

The present investigation was undertaken with 2 main objectives: 1) to determine which varieties in a cosmopolitan collection of wheats change reaction to selected rust races at high temperatures, and 2) to study the rust reaction of representative "temperature-sensitive" varieties in the temperature range where changes in reaction occur.

MATERIALS AND METHODS.—The 87 bread wheats and durums studied were varieties and selections from the world wheat collection distributed in 1953 by the U.S.D.A. for disease testing by cooperators.

Wheat stem rust races 11, 15B, 17, 38, and 56 were used. Cultures of stem rust were periodically propagated, 1 race at a time, on seedlings of Baart wheat (C.I. 1697) in greenhouses containing no other rusted plants. The uredospores produced were collected with a small cyclone collecting device. Spores were stored in screw-cap glass vials at 40°F until used.

Approximately 15 seeds were sown in 4-in. clay pots to provide a minimum of 10 seedlings/pot. The soil mixture (2 parts loam: 1 part peat: 1 part sand) was fumigated in a closed chamber with methyl bromide several days before use. Seeded pots were placed in galvanized iron trays, watered from the bottom, and kept in a rust-free greenhouse at 70°F until inoculated. The primary leaves of most varieties were about 4 in. long when inoculated 7-8 days after seeding.

Plants were inoculated in "dew chambers" by dusting them with uredospore-talc mixtures expelled from small cyclone devices with compressed air. Depending upon the viability of inoculum, about 5-10 mg of spores were thoroughly mixed with 0.5-1.0 g of talc to inoculate 100 pots of seedlings. This much inoculum usually resulted in an average of 10-30 pustules distributed uniformly on the primary leaves of most susceptible hosts.

Inoculated plants were held 18-24 hours in dew chambers controlled to provide very small water drop-

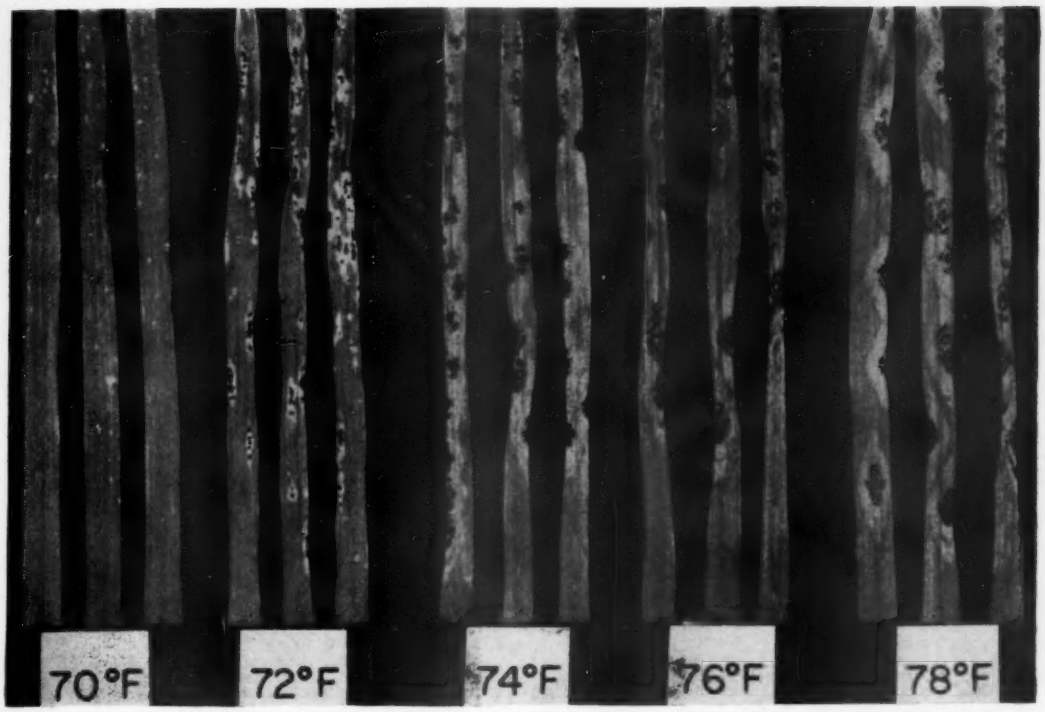

Fig. 1. Lerma 52, 461-6L-4L-2L-1L infected with race 56 and held at 5 temperatures for 15 days after inoculation.

lets, uniformly deposited on all leaf surfaces, at an average air temperature of 70°F (68-72°F) (6). The plants, upon removal from the dew chambers, were immediately transferred to temperature-controlled greenhouse units (5).

The scheme of Stakman, *et al.* (9) was used to record infection types 12-14 days after inoculation.

RESULTS.—*Rust reaction at 70° and 85°F.*—In July, 1953, the reactions of 87 varieties to races 17, 38, and 56 were determined at 70°±1°F and 85°±1°F. With respect to a given race, each variety fell into 1 of 3 categories: category 1—resistant at both 70° and 85°F; category 2—susceptible at both 70° and 85°F; and category 3—resistant at 70° but susceptible at 85°F (Table 1).

The majority of the wheats falling into category 3 were Kenya wheats or wheats with Kenya parentage. McMurachy (C.I. 11876) and the durums Tremez Preto (C.I. 7065) and Tremez Rijo (P.I. 56257 1-1c) were examples of wheats in category 3 (temperature-sensitive varieties) with parentages other than Kenya wheats.

This initial test indicated that temperature-sensitive varieties change reaction somewhere between 70° and 85°F. To narrow this range further, tests were made at 70°, 77°, and 85°F.

Rust reaction at 70°, 77°, and 85°F.—Fifteen varieties of wheat resistant to race 56 at 70°F but susceptible to this race at 85°F in the preceding test were tested to race 56 at 70°±1°F, 77°±1°F, and 85°±1°F in August, 1953, and again in February, 1954, with similar results. All 15 varieties were resistant at 70°F; infection types ranged from 0 to 1 (13 days after inoculation). At both 77° and 85°F, the varieties were susceptible; infection types ranged from 3 to 4.

In similar tests made in August, 1953, and March, 1954, with 20 varieties infected with race 17 and in September, 1953, and April, 1954, with 12 varieties infected with race 38, the plants were resistant at 70°F; susceptible at 77° and 85°F.

These tests showed that the critical temperature inducing change in reaction is between 70° and 77°F.

Rust reaction at 70°, 72°, 74°, 76°, and 78°F.—To determine more precisely the minimum temperature at which changes in reaction occur, tests were made at 70°, 72°, 74°, 76°, and 78°F with races 11, 15B, 17, 38, and 56; and the following wheats: (Egypt Na 101-Timstein) × Mayo 2156-2c-1c-6c; Kenya N.B. 263, J(L), C9968, Cal. 30988 (P.I. 117526); Kenya 291.J.1.I.1 (P.I. 117172); Lerma 52, 461-61-4L-2L-1L; Mayo 52 (E-T-M 2156-6c-1y-2c-1c); and McMurachy (C.I. 11876).

In January, 1955, 5 pots of each of the 6 varieties were placed in a dew chamber and simultaneously inoculated with a given race. Inoculations with each of the 5 races were made on the same day. Following a 24-hour dew period at 70°F, 1 pot of each variety

TABLE 1. Distribution of 87 wheat varieties in 3 temperature-reaction categories for 3 races of *Puccinia graminis* var. *tritici*

Category	Race 17 (%)	Race 38 (%)	Race 56 (%)
Resistant at 70° and 85°F	42	63	55
Susceptible at 70° and 85°F	8	6	8
Resistant at 70° but susceptible at 85°F	50	31	37

inoculated with each race was placed at each of the 5 temperatures.

A typical set of reactions 15 days after inoculation is shown in Fig. 1.

As in the previous tests, predominantly resistant infection types (0; and 1) were recorded for all race-host combinations at 70°F. In certain combinations, occasional pustules larger than those of type 1 were also present but prominent chlorosis and necrosis were associated with such pustules.

Evidence of breakdown in resistance became apparent at 72°F. This was indicated in 1 of 2 ways: 1) a mixture of resistant and susceptible infection types on the same leaf, and 2) completely susceptible reactions, generally infection type 3 but sometimes 3+ or 4. The most common reaction at 72°F was a mixture of both resistant and susceptible infection types on the same leaf.

At temperatures of 74°F and above, the general reaction was susceptibility. Some chlorosis and necrosis were associated with a few of the pustules. An occasional resistant-type pustule was noted, particularly at 74°F, but typically the infection types were in the susceptible class.

Kenya N.B. 263, J(L), and Kenya 291.J.1.I.1. were more resistant to race 38 at 74°F than they were to races 11, 15B, 17, and 56 at this temperature; although at 76° and 78°F, they were quite susceptible to race 38.

At 72°F, susceptibility to race 15B was more common than to races 11, 17, 38, or 56, but this varied with different varieties. Egypt Na 101-Timstein × Mayo 2156-2c-1c-6c, for example, was completely susceptible to 15B at this temperature but mixed infection types were observed on Mayo 52 (E-T-M 2156-6c-1y-2c-1c).

The preceding test was repeated in February, 1955. The trend from resistance to susceptibilty with increasing temperature was similar in both tests. Predominantly resistant infection types occurred at the lowest temperature of 70°F. At 72° and 74°F, there was a shift toward increasing susceptibility. This was apparent in the formation of pustules of both resistant and susceptible types on the same leaf. At 76° and 78°F, the shift toward susceptibility was completed and a preponderance of susceptible-type infections was observed. The exact temperature at which breakdown in resistance occurs and the characteristic pustule type formed in the critical zone, however, vary for different race-host combinations.

A mixed rust reaction was most commonly observed at 72°F in the test made in January. At the next lower temperature (70°F), there was a sharp change to pronounced resistance; at the next higher temperature (74°F), there was an equally sharp change to susceptibility. In the test made in February, this sharpness in change from resistance at 70° to susceptibility at 74°F was less pronounced. Here, the mixed reaction was observed more commonly over the entire range from 70° to 74°F.

CONCLUSIONS AND DISCUSSION.—A mixed reaction was characteristically produced on temperature-sensitive varieties at temperatures intermediate between the highest temperature at which resistance was apparent and the lowest temperature at which susceptibilty was apparent. In 2 experiments involving several races and several varieties, mixed reactions were generally noted at 72-74°F. At temperatures below these values, the reactions were in the resistant class; at temperatures above, the reactions were in the susceptible class. Precise temperature control demonstrated that relatively small differences in temperature, of the order of 2° or 3°F, may shift reactions from 1 class to another.

One cannot, at the present time, generalize too widely concerning temperature-sensitive wheats. A variety which changes reaction to 1 race with increasing temperature may not behave similarly with other races. For example, Kenya 58 × Mida Newthatch II-45-45 was resistant to races 17 and 38 at 70°F and susceptible to them at 85°F but remained resistant to race 56 at both temperatures. On the other hand, the reaction of Kenya 341.0.2.B.1 changed from resistant at 70°F to susceptible at 85°F when infected with race 56 but remained resistant to races 17 and 38 at both temperatures.

From many examples similar to the above, it is apparent that the alteration of reaction is not a direct response to temperature on the part of the host alone since a given host shows alteration in reaction when infected by some races but not others. Neither, apparently, is the alteration of reaction a direct response on the part of the parasite alone since the change in reaction occurs when a given rust race is growing in 1 host variety but not necessarily when growing in another. Thus, it is concluded that the effect of temperature is most likely on the host-parasite complex and not on either of the components independently. Johnson (3) has previously stated this conclusion and elaborated upon it.

This investigation did not provide any fundamental information on the basic problem of why specific hosts infected with specific races produce large susceptible type pustules at 1 temperature and small resistant type pustules at another temperature just a few degrees lower. However, it is suggested that temperature-sensitive varieties would provide excellent experimental material for a study designed to elucidate the biochemical aspects of rust resistance and susceptibility. In a single host variety infected with 1 race, the experimenter can procure both resistant and susceptible host tissue by proper exposure at temperatures only a few degrees apart. Hitherto experiments had to use as a minimum 2 different hosts, or

2 different races, or seedling and adult plants of the same variety in order to procure resistant and susceptible material for comparison.

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MOVEMENT OF NONVOLATILE, DIFFUSIBLE FUNGICIDES THROUGH COLUMNS OF SOIL

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SUMMARY

Some factors that affect movement of fungicides through columns of soil were studied. Soils were uniformly packed in glass cylinders 4 in. in diameter, fungicides were applied in excess quantities necessary to saturate the soil, and samples were assayed biologically for fungicide activity. Soils studied consisted of a Yolo loam, an Oakley loamy sand, sphagnum peat moss, and mixtures of soil, including the U.C. mix (50% peat moss, 50% Oakley loamy sand). Thirteen fungicides were used, but most of the studies were confined to nabam and captan. The movement and penetration of fungicides through soil in columns were related to effects due to soil type, to various soil treatments, and to inherent fungicide factors. All fungicides penetrated loamy sand farther than loam. Peat moss considerably restricted the passage of fungicides. Treatments of soil which made it more porous increased fungicide penetration, especially of those fungicides applied as suspensions. It was possible to leach from the soil all fungicides tested, although some persisted longer than others. The resistance to leaching varied with the types of fungicide and soil. Fungicides penetrated dry soils farther than soils wetted previously, but drained before application. Fungicides prepared as solutions (nabam, Panogen Soil Drench, Vancide 51) penetrated soils more readily than those prepared as suspensions (captan, ferbam, zineb). The penetration of soils by captan was enhanced by formulating the compound with particles 1.05 μ compared with 14.5 μ diameter particles.

Very little is known about the fate of nonvolatile, diffusible fungicides after they are applied to soils in attempts to control plant diseases. Most recommendations for use of these fungicides are based upon empirical knowledge of their persistence or movement in the soil. Some of the factors affecting the persistence of fungicides in soil were reported in an earlier paper (3). This report describes some of the factors that affect the movement of fungicides through columns of soil.

Methods.—Unless otherwise stated, soils were uniformly packed in glass cylinders of 4-in. diameter; fungicides were applied in excess of the quantities necessary to saturate the soil, and samples from portions of the soil columns were assayed biologically (2) for fungicide activity.

Soils used.—Natural and artificially mixed soils were used and prepared on a volume basis. Some of the characteristics of the soils are listed in Table 1.

Soils were dried to a constant weight at temperatures below 50°C. The bulk density $\left(\frac{\text{mean weight}}{\text{mean volume}}\right)$ of the soils was determined by adding approximately 200 ml of dry soil to a 1-liter graduate cylinder, tapping the cylinder on a rubber stopper 5 times from a height of approximately 2 in., and repeating the process until 500 ml of soil were measured. The soil was weighed, and the process repeated 5-10 times with a different sample.

The amount of soil required to pack a cylinder uniformly was determined by multiplying the volume of the cylinder by the bulk density of the soil. The required weight of soil was carefully added and tamped into the cylinder so that it exactly filled the

cylinder to the desired depth. In this way, each column contained the same amount of soil in the same volume and in approximately the same state of compaction (Fig. 1-A).

The moisture content of a 4-in. high column was determined 30 minutes after drainage had ceased. The weight of water in the column then was used to determine the necessary amount of fungicide. The volume of fungicide applied was 150% of the volume of water retained by the 4-in. soil column. In this way, each soil type received the same relative amount of fungicide, although the total quantity varied with each type of soil.

Fungicides used.—Aqueous solutions or suspensions of the following fungicides were used at concentrations of 1,000 ppm (active ingredient) unless otherwise noted: Arasan and Tersan (50 and 75% thiram, *i.e.*, tetramethyl thiuram disulfide); Captan 50-W (50% captan, N-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide); Copper 8-quinolinolate; Dithane D-14 (19% nabam, disodium ethylenebis [dithiocarbamate]); Fermate (76% ferbam, ferric dimethyldithiocarbamate); Lysol (a proprietary disinfectant containing 50% (by volume) of soap, orthohydroxydiphenyl alcohol, and cresylic acid); Panogen Soil Drench (3.5% methyl mercury hydroxide); Parzate (65% zineb, zinc ethylenebis [dithiocarbamate]); Phygon XL (50% 2-3-dichloro-1,4-napthoquinone); Semesan (30% 2-chloro-4-(hydroxymercuri) phenol); Vancide 51 (20% total active ingredients, sodium dimethyldithiocarbamate, and sodium 2-mercaptobenzothiazole); and Vapam 4-S (31% sodium N-methyl dithiocarbamate).

The fungicides were added carefully to the surface of the soil column to avoid channeling. After application and before all of the liquid had drained through, the surface of the soil was releveled to maintain a uniform level surface.

Fungicide assay.—Fungicide activity of soils was determined by the bioassay technique described previously (2). Soil was extruded from the cylinders and cut into sections 1-in. thick. The outer portions of the sections were discarded and the center was used for assaying (Fig. 1-B). In this way, errors arising from channeling and capillary effects along the walls of the cylinder were minimized. Samples of soil then were loosely packed with a spatula into plastic rings (13-mm inside diameter, 13-mm height) and the soil plug was extruded on the surface of an agar plate previously seeded with spores of *Myrothecium verrucaria* (Alb. & Schwein.) Ditm. ex Fr. After 48 hours, the diameter of the clear zone remaining around the soil plugs was measured. The data were expressed as the diameter of the zone of inhibition less the diameter of the soil plug (13 mm). Since the technique depends upon the abilities of a fungicide to diffuse through agar and to kill or to inhibit the test organism, it cannot be used to compare the fungicidal effectiveness of one fungicide with that of another.

RESULTS.—*Effect of soil type on fungicide movement.*—The effect of different soil types on the movement of a number of commonly used fungicides through 4-in. columns of soil was determined. The fungicides were applied to 1 or more of the soils (Table 1). In general, the fungicides prepared in true solution penetrated the 3 soils readily, but those in suspension did not. Also, the effect of soil type on penetration with the solution fungicides was negligible, but quite pronounced with the suspension fungicides. For example, the particles of ferbam, zineb, and Semesan (suspensions) were trapped in the upper 2 in. of the U.C. mix, whereas nabam, vapam, and Panogen Soil Drench (solutions) usually penetrated 4 in. On several occasions, however, captan and Arasan penetrated 3 or 4 in. Captan (suspension) and nabam (solution) were selected for further study, since they were representative of the 2 types of fungicide and the persistence in soil of both had been determined (2).

1) *Penetration through mineral soils in columns.*—Yolo clay loam and Oakley loamy sand (Table 1) were selected as mineral soils suitable for study. The 2 soils were mixed in varying proportions by volume to make a series containing 100, 87, 75, 50, 25, 13, or 0% of the Yolo soil in a Yolo-Oakley mixture. Captan and nabam were applied to these mixtures in columns 4-in. deep. Samples from the top of each 1-in. layer of soil were assayed for fungicide activity. The results of 1 experiment are shown in Table 2. The data substantiate generally the difference between movement of suspension and solution fungicides in soil. The depth

TABLE 1. Characteristics of soils used [a]

Character	Oakley series	Yolo series	U.C. mix
pH	7.40	6.25	5.80
Soil separates (%)			
Coarse sand and larger (over 0.5 mm)	0.5	11.0	Not determined; artificially mixed, using 50% peat moss, 50% Oakley loamy sand by volume. See (1) for details.
Medium sand (0.5-0.25 mm)	17.0	4.3	
Fine sand (0.25-0.10 mm)	61.5	6.7	
Very fine sand (0.10-0.05 mm)	8.0	8.8	
Silt (0.05-0.002 mm)	8.1	41.7	
Clay (less than 0.002 mm)	5.1	27.6	
Textural class	Loamy sand	Clay loam	

[a] The assistance of Dr. O. R. Lunt, Department of Irrigation and Soil Science, University of California, Los Angeles, in making these analyses and in giving advice, is gratefully acknowledged.

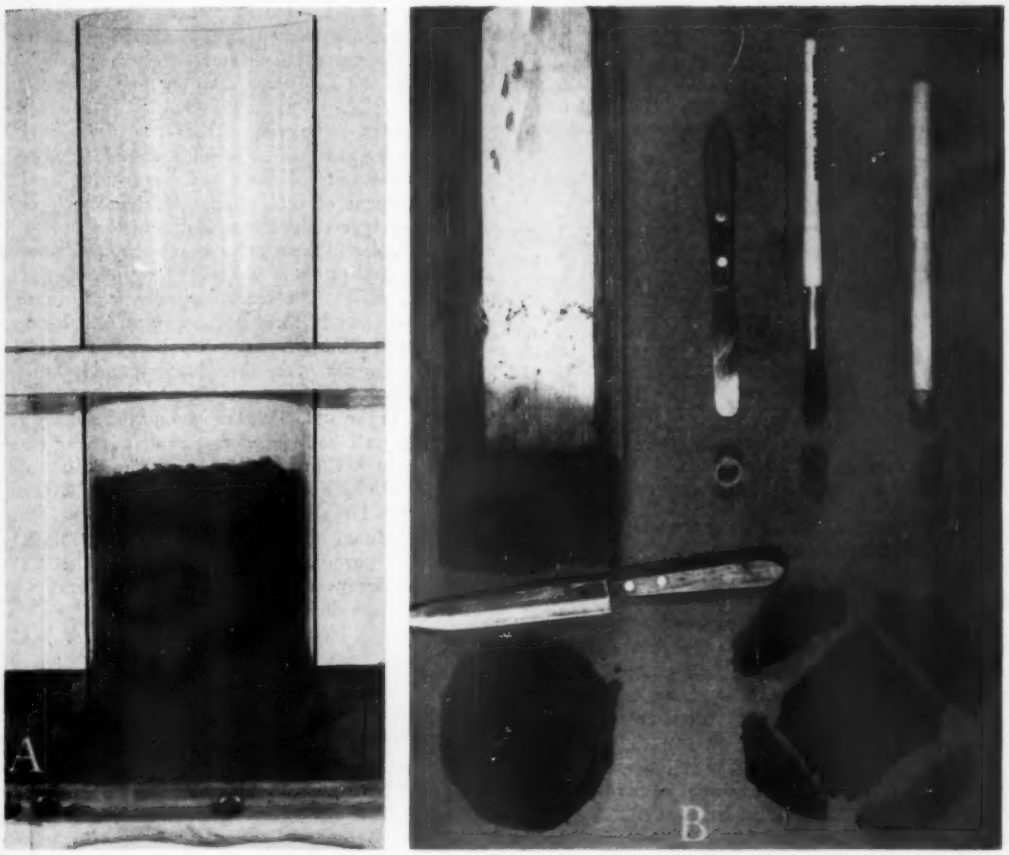

Fig. 1. A) Glass cylinder (4-in. diameter) and rack used to study fungicide movement through columns of soil. A plastic screen on the rack retained the soil, but allowed fungicides to pass freely. **B)** Steps in assaying 1-in. sections of the soil column as described in text.

of penetration of effective levels of the 2 fungicides was affected markedly by the composition of the soil mixture, being greatly restricted by the pure Yolo, but only slightly by the pure Oakley soil. The Yolo loam soil and the Yolo-Oakley mixtures restricted the passage of captan more than that of nabam. With nabam, no appreciable differences in penetration depth existed between mixtures containing 50% or more loamy sand; with captan, equally effective levels at all depths were recorded only with the pure Oakley soil. In the captan series, the drop in fungicide activity obtained at the 3- and 4-in. depths with the mixtures containing 25 or 13% loam appear anomalous when compared with other data. In 2 other experiments of this type, the captan anomalies were confirmed once and refuted once. The nabam data of Table 2 were substantiated in the 2 other experiments. The drop in captan activity in mixtures containing 25 or 13% loam is unexplained.

2) *Penetration through peat moss in columns.*—The penetration of fungicides through previously wet·ed German peat moss in columns 4 in. deep contained in 2-in. diameter glass tubes was studied. Twenty ml of the following fungicides (1,000 ppm active ingredient) were added to the tubes: nabam, ferbam, Tersan, captan (2,000 ppm), Vancide 51, Copper 8 quinolinolate, and Phygon XL. Plugs of peat from various levels in the tube were sampled for fungicidal activity. Only nabam and Vancide 51, which were in solution, penetrated 2 in. of peat. Tersan, a fungicide containing a special wetting agent, was the only suspension preparation that penetrated 1 in., but not 2 in. of peat moss. The remaining fungicides were confined to the top 0.5 in. of the column.

3) *Penetration through U.C. mix in columns.*—The penetration of fungicides through dry U.C. mix consisting of 50% (by volume) Oakley loamy sand and 50% German peat moss is typified by the data presented in Table 3. The individual data show the close correlation between columns and samples in the assay technique. As expected, the penetration through the mixed soil was intermediate between that through peat moss or through Oakley soil alone. The suspension

TABLE 2. Penetration by solutions of nabam or suspensions of captan of Yolo loam or Oakley loamy sand and mixtures of the 2 soils contained in 4-in. diameter cylinders; the volume of fungicide applied was 150% of the volume of water capable of being retained by the soil columns 30 minutes after drainage ceased

Fungicide (active ingredient, ppm)	Depth of soil sample (in.)	Fungicide activity[a]							
	Yolo %	100	87	75	50	25	13	0
	Oakley %	0	13	25	50	75	87	100
Captan 1,000	1	19	19	18	15	18	17	16
	2	16	13	12	13	12	13	14
	3	2	2	7	13	7	7	14
	4	2	4	8	9	5	4	12
Nabam 1,000	1	33	34	36	34	38	35	49
	2	34	37	35	33	33	34	49
	3	21	36	34	35	34	35	43
	4	7	17	24	37	33	34	44

[a] Degree of penetration determined by assaying soils biologically. Fungicide activity = diameter of zone of inhibition — diameter of soil plug used in the assay.

fungicides tended to stay in the upper layers of soil, whereas the solution fungicide readily penetrated 4 in. Of the suspension fungicides, captan penetrated to 4 in., but Parzate was confined to the top 1 in.

Effects due to treatment of soil.—The experiments reported above indirectly showed that the physical properties of soils affect their penetration by fungicides. The effects of steaming, leaching, and moisture content of soils in relation to the soil types were studied in further experiments.

1) *Steaming.*—Since steaming of soil increases water penetration, its effect on fungicide penetration was studied. Soils were steamed 2-3 hours at 100°C, cooled, and packed into glass columns. Adjustments were made for the resulting changes in bulk density and water retention, and appropriate amounts of fungicides were applied as described previously.

Steaming increased penetration of both nabam, a solution; and captan, a suspension. Movement was increased most in Yolo loam, least in Oakley loamy sand, and to intermediate values in the U.C. mix. Greatest increase in penetration due to steaming occurred with captan in the Yolo loam.

The increase in penetration through steamed soils was partially correlated with an increase in the rate of percolation of water through the soil columns. The rate of percolation, however, did not account for all of the increase in movement through steamed soil. Perhaps, the percentage of noncapillary pore space was increased by steaming and was chiefly responsible for the increased penetration of the fungicides. This aspect of the effect of steaming was determined more accurately by studying fungicide penetration through mixtures of varying percentages (by volume) of steamed Yolo and Oakley soils. An excess of nabam (900 ppm) or captan (1,000 ppm) was applied to dry soil in the usual manner. Steaming increased the captan penetration of the various soils about equally, but nabam penetration was affected strikingly. Fig. 2 portrays the activity of nabam at the 4-in. level in the steamed and nonsteamed soil mixtures. The divergence of the curves illustrates the greater effect of steaming on the Yolo loam than on the Oakley loamy sand.

2) *Leaching.*—The effect of repeated applications of water to columns of soil to which fungicides had been applied was studied. Nabam and captan were applied to 4-in. columns of U.C. mix or Oakley loamy sand, both porous type soils. Fungicidal activity at the 1-in. and 4-in. depths in the column was determined immediately after application of 400 ml of tap water and at intervals thereafter. Ten leachings were made through the U.C. mix and 15 through the Oakley soil. Panogen Soil Drench (100 ppm) was applied to the Oakley soil only. Factors other than leaching must be considered which could account for the loss in activity with time in the soil. Previously reported studies (3) in the U.C. mix had shown that captan is very stable in nonsterile U.C. mix, whereas nabam is unstable. Nabam's activity decreases rapidly to a low level and then persists for a long time. The persistence curves of these 2 fungicides are included in Fig. 3, 4 for comparative purposes. Spanis (4) showed that Panogen in soil decays biologically and that the curves are similar to those shown (3) for Semesan.

A number of facts are apparent in Fig. 3, 4. Nabam was leached very rapidly from the Oakley soil, but

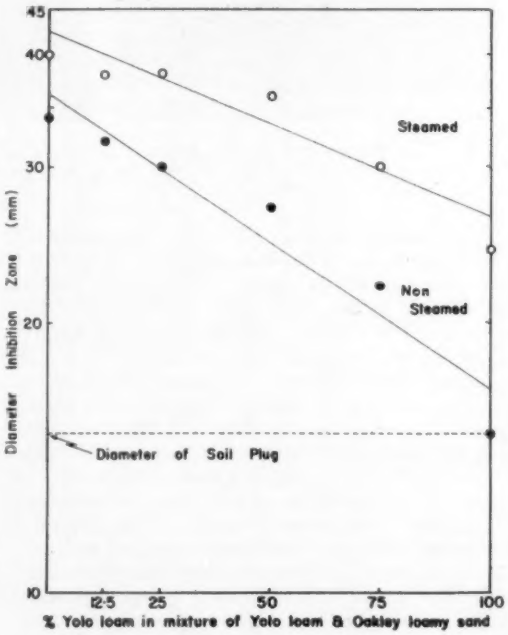

Fig. 2. Effect of soil steaming on nabam penetration of columns of soil containing various proportions of Yolo loam and Oakley loamy sand. Data are presented showing the fungicide activity at the 4-in. depth.

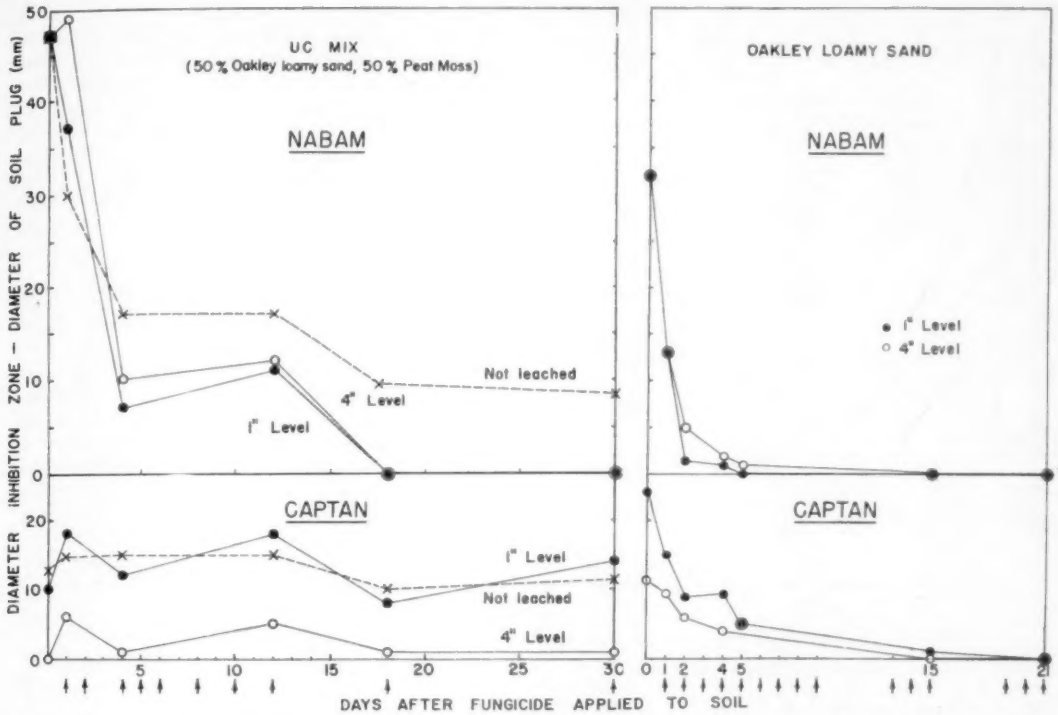

Fig. 3. Effect of leachings of water on the movement of nabam and captan in U.C. mix and Oakley loamy sand in columns. Arrows indicate days on which 400 ml of tap water were added.

surprisingly slowly from the U.C. mix. In the Oakley soil, activity was almost nil at the 1- and 4-in. levels after 3 leachings, whereas it was relatively high in the U.C. mix after 8 leachings. The reaction of captan was considerably different in the 2 soils. With the Oakley soil, it required 5 leachings to move the fungicide to equal concentrations at the top and bottom of the column; by the 12th leaching, most of the captan had been removed from the soil. With the U.C. mix, the concentration of the top 1 in. was consistently greater than the bottom 4 in. of the column; neither was changed appreciably even after 10 leachings. Panogen Soil Drench, a solution fungicide, was remarkably resistant to leaching in the Oakley soil (Fig. 4), although a great deal of the toxicant was lost in 2 leachings. Unfortunately, it was not used on other soils. Although Panogen is decayed in soil by microorganisms (4), the concentration of 100 ppm used probably was sufficient to eradicate most of the soil organisms throughout the soil column so that biological decay was not a major factor in these studies. After the 1st leaching, the concentration of Panogen in the bottom of the column was consistently higher than at the top. The reason for this is unknown.

3) *Surface tension depressants.*—In several experiments, the effect of duPont Spreader-Sticker additive (sodium sulfates, mixed long chain alcohol fatty acids and esters, 88%) on penetration of soils by fungicides was investigated. In early experiments, it appeared that Tersan (75% thiram), which is formulated as a wettable powder, penetrated peat moss columns better than its counterpart, Arasan (50% thiram), which is not "wettable." The addition of the spreader sticker to Semesan, Tersan, or zineb, however, did not increase penetration through a column containing peat moss or the U.C. mix.

4) *Moisture content of the soil.*—Most of the fungicides, when applied in excess, penetrated dry soil better than soil which had been wetted and drained at least 1-2 hours. Penetration by nabam was slightly affected, but penetration by captan was affected markedly. For example, captan penetrated a column containing wet U.C. mix only 2 in., whereas it penetrated a dry column 4 in. The effect with Yolo loam was similar, but less pronounced. Differences in penetration of wet and dry soil, as well as different kinds of soils, are probably due to the rate of water percolation through the soils. Water penetrates the dry soils much faster than wet, drained soils, especially soils with a high clay content. The faster the percolation the less the chance of suspended particles of fungicide settling out on the soil particle surfaces, and the deeper the penetration. With fungicides in solution, the rate of percolation is relatively unimportant; thus, there is

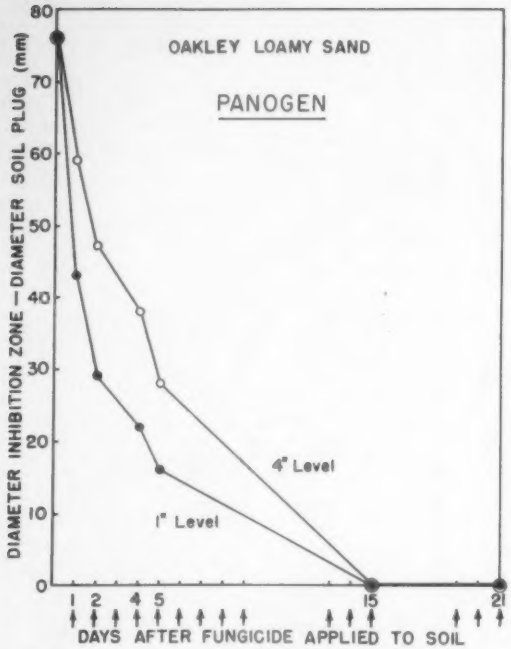

Fig. 4. Effect of leachings of water on the movement of Panogen Soil Drench in Oakley loamy sand in columns. Arrows indicate days on which 400 ml of tap water were added.

less difference between the penetration of wet *vs.* dry soils.

Effects due to fungicides.—1) *Solution vs. suspension fungicides.*—Differences have been noted in penetration of fungicides which are applied as solutions and those applied as suspensions. All of the fungicides which are true solutions (nabam, vapam, Vancide 51, Lysol, Panogen Soil Drench) penetrated soils much deeper that the fungicides prepared as suspensions.

2) *Relation of size of particles of fungicide to penetration of soil.*—Since soil may act as a filter in preventing suspended fungicides from penetrating soil, the effect of particle size on penetration was studied. Captan formulations prepared by the Stauffer Chemical Company containing particles 14.5, 2.9, 2.7, 1.5, and 1.05 μ in diameter were used. When applied to a dry U.C. mix, all preparations penetrated through a 4-in. column to the same degree, but with moist Oakley soil pronounced differences were noted (Fig. 5). The penetration by suspensions containing captan particles of 2.9 μ or less was similar; data for the 1.05 μ diameter captan preparations were representative of the group. There were no differences in degree of penetration of either the Yolo or Oakley soils with captan containing particles 14.5 μ in diameter. The great differences occurred with captan made up of particles 1.05 μ in diameter applied to Oakley loamy sand. The coarse-textured Oakley soil retained the large particles of captan (14.5 μ), but did not materially retain the smaller particles.

3) *Volatility effects.*—These studies were confined to nonvolatile fungicides as contrasted to the gaseous fumigants whose activity, in part, is dependent upon their low boiling points and resultant high volatility. Semesan and Lysol, however, were somewhat volatile. Gases from these fungicides caused the surface of the *Myrothecium* colonies to turn gray when soil plugs containing the fungicides were bioassayed. In further experiments, the inhibition zones obtained in gas-tight petri dishes seeded with *Myrothecium* were similar to those obtained in unsealed dishes. As a result, it is unlikely that the low rate of volatility of these compounds materially affects their effective penetration of soil.

Discussion.—Several precautions should be taken in relating these studies to the field. Probably, the fungicide behavior will be similar in the field and in the laboratory, providing fungicides can percolate through the soil. Poor drainage, however, often closely allied to root disease problems, may mitigate the effectiveness of these fungicides and should be a primary consideration in applying these laboratory findings to the field. These results are based on the assay method used, but they have not been related to the effectiveness of fungicides in controlling various soil-borne pathogens in the soil. Such studies are currently under way.

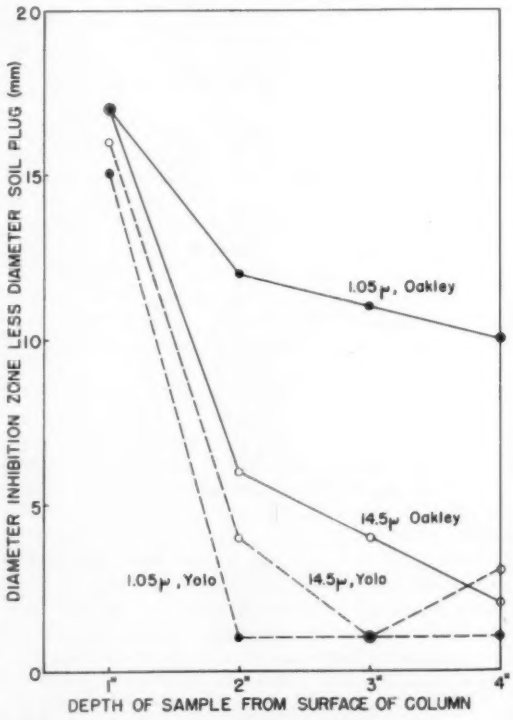

Fig. 5. Effect of size of captan particles on penetration of columns of Yolo loam and Oakley loamy sand soils.

TABLE 3. Penetration by solutions or suspensions of fungicides of dry U.C. mix (50% Oakley loamy sand, 50% German peat moss) contained in 4-in. diameter columns; the volume of fungicide applied was 150% of the volume of water capable of being retained by the soil columns 30 minutes after drainage ceased

Fungicide (active ingredient, ppm)	Column no.	Fungicide activity at depth indicated[a]															
		1 in.				2 in.				3 in.				4 in.			
		Sample no.			Mean	Sample no.			Mean	Sample no.			Mean	Sample no.			Mean
		1	2	3		1	2	3		1	2	3		1	2	3	
Nabam, 200	1	17	16	17		14	14	14		7	11	11		6	9	6	
	2	16	17	16		14	13	12		5	11	9		6	4	6	
	3	18	17	19		16	15	15		8	9	12		1	5	4	
					17.0				14.1				9.2				5.2
Arasan, 1,000	1	26	27	26		4	4	4		0	0	0		0	0	0	
	2	23	26	26		18	18	18		4	9	11		1	1	1	
	3	27	24	26		16	19	17		8	9	10		1	1	1	
					25.7				13.1				5.7				0.7
Captan, 1,000	1	18	15	21		9	8	8		5	6	4		1	1	1	
	2	14	11	12		10	8	8		6	4	5		6	6	4	
	3	11	11	11		6	7	6		4	3	4		1	1	1	
					13.8				7.8				4.6				2.4
Parzate, 1,000	1	9	9	9		0	0	0		0	0	0		0	0	0	
	2	9	10	12		0	0	0		0	0	0		0	0	0	
	3	6	7	8		0	0	0		0	0	0		0	0	0	
					8.8				0.0				0.0				0.0

[a] Diameter of zone of inhibition — diameter of soil plug used in the assay.

Further, the diameter of inhibition zones of assay organism growth cannot be used to compare the fungicidal value of 1 fungicide with another. The zone of inhibition depends on the inherent toxicity of the fungicides to the specific organisms being used in the assay; also, it is a measure of the solubility and diffusibility of the fungicide in agar. The solution fungicides invariably produce larger zones of inhibition than the suspension fungicides, but they are not necessarily more potent fungicides.

Moreover, fungicides may be formulated specifically for use in the soil, just as most are formulated presently for use on plant foliage. If the penetration of soil by captan can be increased with particles of small diameter, perhaps penetration by other fungicides also can be increased. Probably, the smaller fungicide particles would be more active in soil, and fungicide effectiveness would be increased. These and other factors may develop if the fungicides are formulated expressly for use in the soil.

Several principles of the behavior of nonvolatile soil fungicides in soil appear capable of being formulated at this time. Fungicides which are applied as solutions will penetrate soils farther than fungicides applied as suspensions. Fungicides will penetrate coarse soils farther than fine-textured soils. Sphagnum peat moss in soil will restrict the passage of fungicides considerably, probably by sorption processes. The clay fraction in soils restricts the passage of fungicides more than the coarser fractions. The depth of penetration in soil is related to the rate of percolation of water through the soils; the faster the rate, the farther the penetration. Fungicides will penetrate dry soils farther than wet soils. The penetration ability of a soil fungicide applied as a suspension may be enhanced by formulating the fungicide out of smaller particles. Solution fungicides may be leached more readily from soil than suspension fungicides. Although solution fungicides are leachable from soil, they may be remarkably persistent and be retained by soils after many flushings with water. To evaluate the factors affecting movement of fungicides in soil, the persistence behavior in the absence of leaching must be known. These generalizations are admittedly broad and many may not be universally applicable, but they are presented with the aim of stimulating further thought and experimentation.

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COMPARATIVE PATHOGENICITY OF SCLEROTIUM ROLFSII AND RHIZOCTONIA SOLANI TO SPANISH PEANUT

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SUMMARY

A study of the comparative pathogenicity of *Sclerotium rolfsii* and *Rhizoctonia solani* was made under greenhouse conditions. Sites of infection appeared to be quite different for the 2 fungi. *S. rolfsii* was a more vigorous pathogen of branches than the other fungus. Until near time of plant maturity, it also was more destructive to peg tissues than *R. solani*. On the other hand, *S. rolfsii* invaded few tap roots but *R. solani* caused extensive crown and root injury throughout the life of the plant. A yield-reducing potential of 25% was indicated for the latter fungus. *R. solani* induced lesions on fruits that were similar if not identical with those of *Pratylenchus brachyurus*. *R. solani* was seed-borne to a substantial degree; under temperature conditions of 21-24°C, it reduced seedling emergence approximately 30% with a total infection after 3 weeks of 40-70%. In contrast, less than 10% of the plants infected by *S. rolfsii* bore infected fruit.

INTRODUCTION.—A *Rhizoctonia* disease of peanuts in India was described in 1912 (21). As of 1914, Wolf (30) reported it unknown in the southeastern United States where peanuts had been grown in large volume for about 50 years. Since that time *Rhizoctonia* spp. have been associated with root, stem, and crown rot of peanuts many times (1, 5, 6, 8, 10, 11, 13, 14, 15, 20). They also have been isolated from peg lesions (8, 10, 28), from undescribed lesions on immature and mature pods (1, 2, 8, 11, 17, 19, 20, 21), and from kernels (2, 7, 8, 9, 12, 16, 17, 27).

Rhizoctonia solani Kühn is recognized as a pathogen of peanut seedlings, but its role as a pathogen of older plants is not well understood. Sundararaman (26) reported 40% reduction in seedling emergence due to seed-borne infection by the fungus. Poole (17) and Evans and Poole (8) showed that at least 2 strains of the fungus invaded seeds under field conditions. In laboratory tests, 1 strain was most virulent at 18°C and the other at 28°C. Crosier (7) reported that germination tests with several Southern-grown lots of seed could not be completed, when pregermination seed treatment was neglected, due to seed-borne *Rhizoctonia* spp. and other fungi. Garren and Wilson (10) reported that *R. solani* was isolated only rarely from older plants exhibiting symptoms of collar or crown rot, but was the only fungus present that was definitely pathogenic to the plant. No other pathogenicity experiments are reported. Garren and Wilson (10) concluded that "At present any theory as to *R. solani* and diseases of peanuts beyond the seedling stage is purely suppositionary."

In Texas, as elsewhere, *Sclerotium rolfsii* Sacc. has been considered, with some limited exceptions, the only root, peg, and stem rotting fungus of consequence. Shaw (23) questioned whether *Rhizoctonia* spp. were involved in the root-rot complex of peanuts in North Carolina. Poole (17) concluded that *R. solani* was responsible for part of that complex based upon his work with infected seed. Shaw and Hebert (24) indicated that *Rhizoctonia* was the most important root pathogen in North Carolina in 1942. It has been of some importance in Oklahoma (13, 14) and of apparent minor importance in Florida (20). Since 1958, *R. solani* has been particularly common in the older peanut-growing areas of Texas. It has caused damage in fields where gramineous plants, such as sorghum and corn, have been grown for 2 years between peanut crops.

This report is based upon a comparative study of the pathogenicity of *R. solani* and *S. rolfsii* to Spanish peanuts, made in the greenhouse, from the seedling stage to plant maturity. It attempts to clarify the role of the 2 fungi with regard to seedling mortality, stem and root infection, and peg and pod infection.

MATERIALS AND METHODS.—Plants were grown to maturity in 8 in. deep greenhouse benches. Soil used in the experiments was a fine sandy loam from areas lacking a history of peanut culture. Soil was disinfested with chloropicrin unless otherwise stated. Chloropicrin was injected to a depth of 6 in. in 1.5-ml portions in each corner of 6-in. grids, marked out over the surface of the benches. Injection holes were closed immediately. As a bench was finished, its surface was watered to reduce the rate of vapor loss. Fumigated benches were sealed with plastic sheets for approximately a week; then, soils were aerated 3-4 weeks before planting. Minimum soil temperatures were maintained at 29-32°C by heating cables in the bench bottoms.

Seeds, untreated in the 1959 experiment and Thiram-treated in the 1960 experiment, were planted 2-in. apart in rows 6-in. apart. Each replication consisted of 96 seeds; there were 6 replications for each of 4

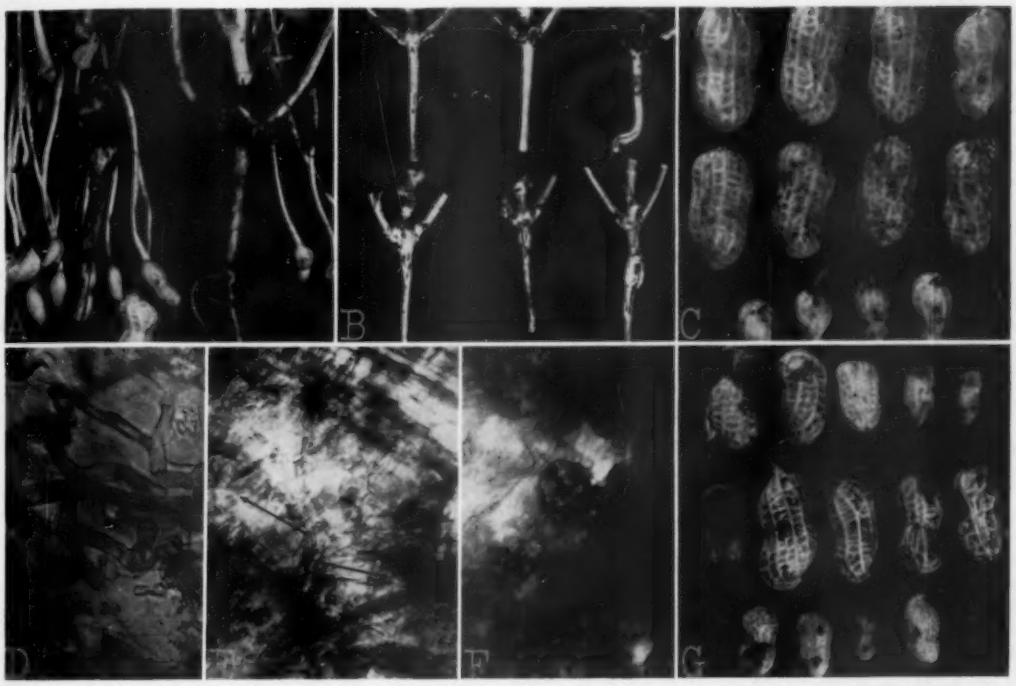

Fig. 1. A) 90-day-old peanut plants; *Rhizoctonia solani* infected plant, left; and *Sclerotium rolfsii* infected plant, right. **B)** 120-day-old plants; *S. rolfsii* infected plants, top row; *R. solani* infected plants, bottom row. **C)** Pods from greenhouse-grown 90-day-old *R. solani* infected plants. **D)** *R. solani* mycelium within a pod lesion of a greenhouse-grown infected plant. **E)** Section from a pod lesion of a field-infected plant containing *R. solani*, upper arrow; and *Pratylenchus brachyurus*, lower arrow. **F)** *R. solani* mycelium and sclerotia on and within the seedcoat of a fruit from a greenhouse-grown infected plant. **G)** Pods from greenhouse-grown 120-day-old plants: *S. rolfsii* infected pods, upper row; *R. solani* infected pods, center and lower rows.

sampling dates, 30, 60, 90, and 120 days after planting. The latest time is generally considered normal for crop maturity, although it is commonly harvested at various lengths of time. The data presented in this report are mean reactions of several similar lines of Spanish peanut (*Arachis hypogeae* L.) to infection by the 2 pathogens.

R. solani and *S. rolfsii*, from hyphal-tip isolates, were grown separately on moist, autoclaved, whole oats under aseptic conditions for about 2 weeks. The fungus-impregnated oats were then air-dried and ground to pass a 20-mesh screen. Ground inocula were mixed thoroughly with steam-sterilized sand at the rate of 0.2% by weight. The sand-inoculum mixture was added to the newly planted benches in a ½-in. layer after the seeds were planted. Soil of the control benches was not inoculated.

Plant counts were made at 15, 30, 60, 90, and 120 days. An infection index, based upon severity of crown and taproot infection, was calculated at each sampling date. Plants scored as 0 were macroscopically free of infection; those scored as 3 were dead. At the 60-, 90-, and 120-day dates, measurements were made of the number of fruits/plant, the percentage of plants with infected pegs, and the percentage of plants with infected pods. Isolations were made from the margins of lesions from infected plant parts at each harvest to verify presence of the inoculated pathogen with a particular type of injury.

Plant emergence and stands.—Stands of plants in the *S. rolfsii* and control series were approximately the same. Percentages of emergence were 96-98% at the 15- and 30-day counts. Their stands at maturity were 98.8%. On the other hand, emergence of seedlings was reduced in the *R. solani* series. Stands were highest at 15 days (82%), dropping to 75% at 30 days, and to 70% at 120 days. The drop in stand between 30 and 120 days was not statistically significant. Under the conditions of this study, most plants were lost prior to emergence.

Taproot, crown, and stem infection.—For each of the 1st 3 counts, the disease index was considerably higher for the *R. solani* series than for *S. rolfsii*. It was 1.92, 1.59, and 1.61, respectively, at 30, 60, and 90 days for *R. solani;* and 0.95, 1.10, and 1.21, respectively, for *S. rolfsii*. The 30-day infection index was higher than the 60- and 90-day indexes in the *R. solani* series, largely due to pre-emergence damping-off and to a lesser amount of postemergence damping-off between emergence and 30 days. Fig. 1-A illustrates

the severe cankers and distortion of the taproot and crown due to infection by *R. solani* and the more moderate crown cankers caused by *S. rolfsii* on 90-day-old plants. The infection indexes for both pathogens were about equal by 120 days after planting, respectively, 1.84 and 1.86 for *R. solani* and *S. rolfsii*. This study indicated that the sites of infection for the 2 pathogens were distinctly different. All taproots were severely cankered in the *R. solani* series; the roots and crown were distorted, due to wound tissue development, but crown branches were relatively free of infection. On the other hand, only occasional taproots were invaded by *S. rolfsii*, though by 120 days the crown branches were uniformly and severely injured as a result of multiple infections by that fungus (Fig. 1-B).

The number of developing pods/plant.—The number of pods that developed on a plant basis with respect to time and treatment varied considerably. The total number of potential pods/plant was determined at the last 3 harvest dates by counting all of the intact elongating pegs with developing pods as well as maturing pods below the soil surface (Fig. 2-A). There was little increase in the number of pods formed in the control series after 60 days, even though the plants continued to blossom after 90 days. The number of pods/plant did not increase in any of the treatments at 90-120 days. There were fewer pods/plant at all dates in the *S. rolfsii* series. Equal numbers of pods were present in the control series and the *R. solani* series at 60 days. At 90 and 120 days, however, there were 4 more pods/plant in the *R. solani* series than in the control series.

Influence of *R. solani* upon yield was measured, since plants infected by that fungus in the seedling stage produced more fruits than did healthy ones. Three replicates of 100-g samples of fresh pods from both healthy and infected plants were shelled and the kernels were allowed to air-dry. The total number of kernels, the number of sound and shriveled kernels, and their respective weights were recorded. A 25.7% decrease in total weight of sound kernels was found in the *R. solani* series. The reduction was due to the large number of small, shriveled kernels in that treatment (Table 1).

TABLE 1. Comparative yields of air-dry kernels from 100-g samples of fresh fruit of healthy peanut plants and plants infected with *Rhizoctonia solani*

Treatment	No. of kernels			Weight of kernels (g)		
	Total	Sound	Shriveled	Total	Sound	% decrease in sound kernels
Control	142	124	18	40.2	37.7	—
R. solani	148	95	53	33.3	28.0	25.7
LSD, 0.05	ns	16.9	21.7	4.5	3.3	—

Peg and pod infection.—The number of plants with infected pegs and the number of plants with infected pods were counted at each sampling date. Prior to 90 days after planting, *S. rolfsii* infected the pegs of more plants than did *R. solani*; by 120 days, all plants, regardless of pathogen, had infected pegs (Fig. 2-B). Although all plants in the *R. solani* series bore some infected pegs at maturity, the number of infected ones was greater in the *S. rolfsii* treatment. The lesions induced on pegs by the 2 fungi were indistinguishable. On the other hand, the lesions on pods were recognizably different (Fig. 1-C, G). Shell lesions caused by *S. rolfsii* were orange-yellow to light tan in color on young pods and were light brown to black, with a target spot appearance, on older pods. Those caused by *R. solani* were dark brown and angular from the beginning. In some instances, the lesions had a raised appearance due to the presence of fungus sclerotia on the surface. Lesions were found on pods of all ages in both treatments, but *R. solani* was by far the more effective pod invader (Fig. 2-C). By 120 days after planting, essentially all fruits of all plants were infected by *R. solani*, but fewer than 10% of the plants in the *S. rolfsii* series had infected fruits.

The lesions that *R. solani* induced on the shells of fruits in repeated greenhouse experiments were similar, if not identical, with those attributed to the root lesion nematode, *Pratylenchus brachyurus* (Godfrey, 1929) Goodey, 1951 (11, 25). When this similarity in lesion morphology was observed in a 1959 experiment, numerous lesions were examined microscopically for presence of the nematode. The results were always negative and *R. solani* was reisolated on agar media from a high percentage of lesions; typical mycelium of the fungus was always found at the margins of lesions (Fig. 1-D). The experiment was repeated with the same results in 1960. It is clear that the fungus invades the shells of pods in the absence of *P. brachyurus*. Good, *et al.* (11) reported that "Colonies of miscellaneous microorganisms, including *Rhizoctonia*, developed on nutrient agar plates inoculated with root and shell tissue from both infested and non-infested pots." *R. solani* was readily isolated on agar from surface-disinfected, greenhouse-grown material. On the other hand, a much lower rate of recovery was experienced with material collected from the field. Table 2 data are based on isolations from the margins of lesions from field and greenhouse sources. Tissue was surface disinfected for 1, 2, and 4 minutes in 1:10 dilutions of 5.25% sodium hypochlorite and incubated on acidified PDA. Isolations were made from 100 lesions for each treatment. When nonsurface-disinfected field material was cultured on distilled or tap water, the frequency of recovery was higher (19-28%) than from agar isolations.

Numerous hand sections from lesion margins of infected shells of field-grown plants were stained with cotton blue and examined microscopically. Nine samples from 3 areas of peanut production in Texas were studied. In all instances, the fungus was isolated from a low percentage of lesions, but mycelium, apparently *R. solani*, was present in the marginal cells of all lesions (Fig. 1-E). *P. brachyurus* also was present in the lesions from 8 of the 9 samples; only *R. solani* and other fungi were in the other sample.

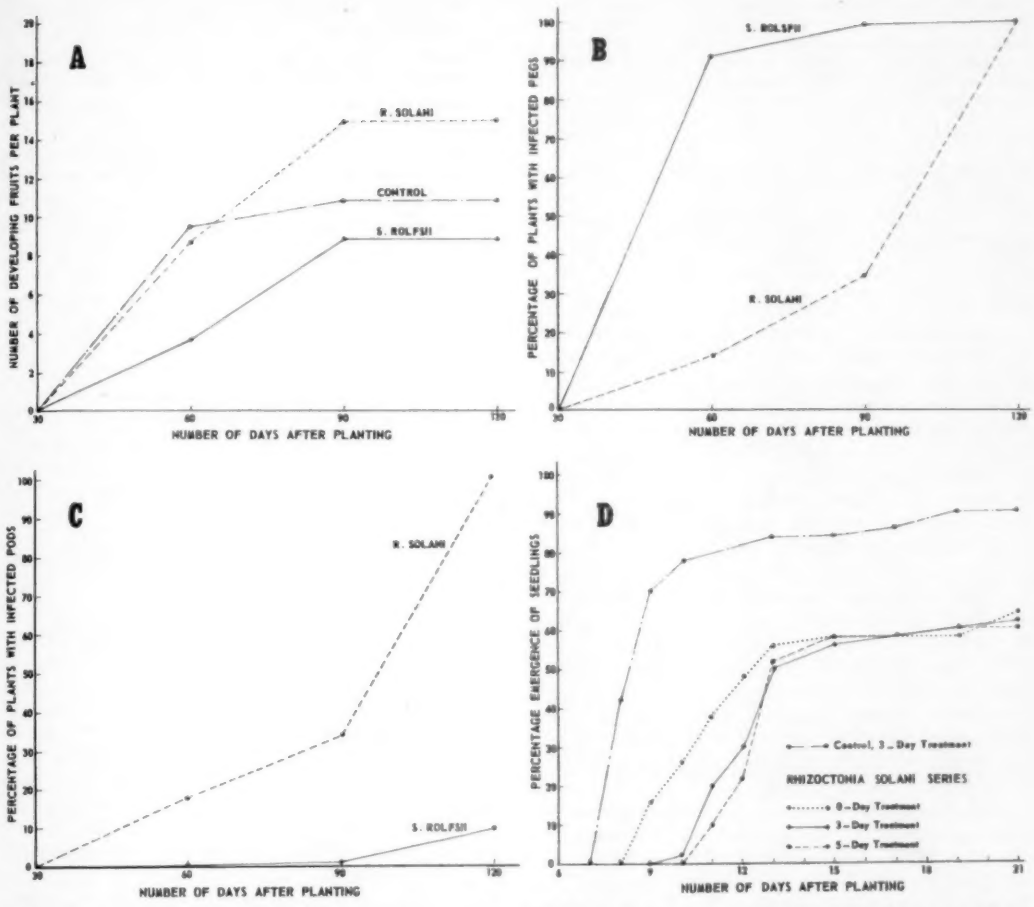

Fig. 2. Influence of *Sclerotium rolfsii* and *Rhizoctonia solani* on growth and development of Spanish peanut plants. **A)** Number of developing fruits/plant. **B)** Percentage of plants with infected pegs. **C)** Percentage of plants with infected pods. **D)** Influence of seed-borne *R. solani* on emergence of Spanish peanut plants in sterile soil at 21-24°C.

Seed infection.—The development of seed infection was observed in these experiments. When infected pods of greenhouse-grown plants were harvested 120 days after planting and dried immediately, the kernels were macroscopically free of infection. When fresh pods were held in closed plastic bags for 3-5 days after harvest, obvious invasion of the seed coat was observed. Mycelium of the fungus was found within the seed coat and mycelium and sclerotia of the fungus were found on its surface (Fig. 1-F). In contrast to healthy seed, infected seed was light tan instead of pink. Necrotic fungus-invaded areas were particularly obvious in seeds from pods that were not allowed to dry until 5 days after harvest.

The influence of seed-borne *R. solani* upon the rate of emergence, total stand, and amount of infection was measured in 2 greenhouse trials. In each, a control

TABLE 2. Percentage recovery of different fungi from margins of lesions excised from shells of fruit of mature greenhouse- and field-grown Spanish peanuts; lesions surface sterilized 1, 2, and 4 minutes in a 1:10 dilution of 5.25% sodium hypochlorite; 100 lesions for each treatment

	% recovery of fungi					
	1 min.		2 min.		4 min.	
	Field	Green-house	Field	Green-house	Field	Green-house
No growth from tissue	16	2	52	26	47	22
Rhizoctonia solani	9	70	4	53	0	45
Macrophomina phaseoli	6	0	4	0	4	0
Fusarium moniliforme	28	0	10	0	15	0
F. oxysporum	20	24	13	17	10	24
Penicillium spp.	18	4	15	4	18	9
Aspergillus niger	0	0	0	0	1	0
Aspergillus spp.	3	0	2	0	4	0
Trichoderma viride	0	0	0	0	1	0

TABLE 3. The influence of temperature upon stand and development of infection due to seed-borne *Rhizoctonia solani* in Spanish peanut seed from infected fruit stored in a saturated atmosphere for 0, 3, and 5 days after harvest; seed for control series from plants grown in noninfested soil

	% infection and stand after 3 weeks			
	21-24°C		21-43°C	
Treatment	Infection	Stand	Infection	Stand
Control	20.0	90.0	2.7	74.0
0-day	38.9	64.0	29.7	72.0
3-day	63.1	62.0	51.3	72.0
5-day	71.3	60.0	81.0	66.0

series of seeds from plants grown in noninfested soil and not allowed to dry for 3 days was compared with seeds from infected fruit dried immediately after harvest, and from fruit not allowed to dry until 3 and 5 days after harvest. Fifty seeds were planted, 1/pot with steam-sterilized soil for each of the 4 treatments. One trial was grown in the greenhouse during September when temperatures were 21-43°C. Under those conditions, the seedlings began to emerge on the 3rd day and stands were near maximum within 6 days. Total stands, regardless of treatment, were 66-74% 3 weeks after planting. The 2nd trial was grown in a refrigerated-growth chamber where temperatures were 21-24°C. The rate of emergence was lower than at the high temperature (Fig. 2-D), but total stand in the control series was considerably higher (90%). Total emergence in the other treatments was 29-33% less than in the control series. After 3 weeks, plants were washed from the pots and examined for taproot and hypocotyl infection. Total infection and stand percentages appear in Table 3.

A small amount of infection was evident in control plants of the high-temperature series and a greater amount (20%) was present in the low-temperature experiment. Greenhouse benches remained moist in the low-temperature experiment; thus, increased infection in the control series may have been due to contamination between soil containers. Seeds from obviously infected pods produced a high proportion of infected plants. Approximately 25% had infected apical meristems and injured leaves. At 21-24°C, most seeds began to germinate, but the hypocotyls were destroyed shortly after they began to elongate. This was not evident in the high-temperature experiment where the control series emerged poorly. *Penicillium* spp. and *Aspergillus* spp. were isolated from the decayed hypocotyls but their significance is unknown. The exceptionally high temperatures in the experiment are believed primarily responsible for the over-all poor emergence in the control series. Results indicated that many seeds of the 0-day treatments were infected even though infection was not macroscopically evident.

DISCUSSION.—*S. rolfsii* did not contribute to stand depletion under the conditions of this study, although it is recognized that the organism does kill young plants under field conditions, particularly when plants are partially covered with moist, infested soil during early cultivations. The amount of branch mortality would no doubt have been greater also if the experiments had been more comparable to field conditions. Results did indicate, however, that *S. rolfsii* was a more vigorous pathogen of branches, regardless of age of the plant, than *R. solani*. The latter fungus caused approximately 20% reduction of emergence within 15 days after planting; the final stand was about 70% of the control series when Thiram-treated seeds were planted in artifically infested soil.

S. rolfsii, until near plant maturity, was much more virulent to pegs than *R. solani*, but it was less than 10% as effective as the other fungus in causing injury to shells. This appears to agree with kernel isolation data of other workers. Prince (18) reported 2.7% invasion of kernels whereas other investigators reported only trace amounts of kernel infection (3, 8, 12, 16, 29). *R. solani* infected essentially all shells; and 70-80% seed-borne transmission of the fungus was observed in the greenhouse-transmission experiments. At 21-24°C, approximately 30% seedling mortality resulted from such infected seeds. Apparently, kernel infection was increased under conditions that did not favor rapid drying of fruit at harvest time. These results indicate that kernels, macroscopically free of infection, may harbor the fungus. Therefore, it would be risky to plant kernels from infected pods.

In repeated greenhouse experiments, using chloropicrin-sterilized soil, lesions caused by *R. solani* on pods were similar to, if not identical with, those attributed to *P. brachyurus*. The latter organism was never observed in greenhouse-produced material. Microscopic examinations and isolations from field material, of similar appearance, revealed that both organisms were present in 8 of 9 samples; only *R. solani* and other fungi were associated with 1 of 9 samples from 3 areas of production. *Macrophomina phaseoli* (Maubl.) Ashby also induces lesions on peanut pods, similar to those caused by *R. solani*. Both sclerotia and pycnidia of the fungus have been found in shells of fruits collected in Texas. Shaw (21) illustrated the disease in 1912 and attributed it to infection by *R. solani*; a later report (22) indicated the fungus was *M. phaseoli*. Bouriquet and Jaubert (4) showed that *M. phaseoli*, like *R. solani*, could be seed-borne within the testa of kernels. In light of this evidence, the role the nematode plays in causing fruit necroses, described by Steiner (25) and Good, *et al.* (11), needs clarification. This belief is strengthened by the latter authors' report that the fungus was associated with their experiments, although apparently isolated infrequently. In these experiments, the fungus was recovered from approximately 70% of the lesions from greenhouse material and from 9-28% of the lesions from field material.

Only occasional taproots were invaded by *S. rolfsii*; plant injury was primarily at stem and peg sites, where the fungus was extremely active. *R. solani* was a vigorous taproot invader that caused severe crown and taproot cankers with consequent development of distorted taproots and rough swollen crowns as wound

tissue formed in response to infection. This girdling effect may have been responsible for the increase in number of fruit over the number produced in the control series. Although the potential number of fruit/plant was greater in the *R. solani* infected plants, the amount of sound kernels produced was about 25% less than the control. The fungus appears to be a vigorous pathogen throughout the life history of the peanut plant, causing both stand depletion and direct yield reduction.

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ECONOMIC SIGNIFICANCE OF BEET WESTERN YELLOWS (RADISH YELLOWS) ON SUGAR BEET

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SUMMARY

Beet Western yellows (previously described as radish yellows), incited by a virus capable of inducing foliage yellowing of sugar beet indistinguishable from that induced by isolates of the beet yellows virus, is widespread in beet plantings in California and Oregon. Indexing has indicated that beet Western yellows virus may be more prevalent in these areas than the beet yellows virus.

Replicated field tests on sugar beets have indicated that isolates of the Western yellows virus are capable of inducing serious losses in sugar yield. These losses apparently are additive to losses produced by the beet yellows virus when the viruses occur simultaneously.

The widespread occurrence of the Western yellows virus in California sugar beet plantings, coupled with its effect on sugar yield, indicates the need to include studies of resistance to this virus as well as to the beet yellows virus in a breeding program.

INTRODUCTION.—The complex of sugar beet diseases, known as yellows, is extremely common in California. By the end of the season, in many growing areas, most fields show high percentages of infection. One of the components, a yellowing disease of sugar beet and other crop and weed plants, previously described as radish yellows (3), has not been studied extensively in regard to the damage caused under field conditions. This disease on sugar beet, indistinguishable from foliage yellowing induced by isolates of the beet yellows virus, has been found widespread in California beet plantings.

Widespread distribution of this disease on sugar beet in Western growing areas, coupled with other data reported in this paper regarding the economic significance of the disease, suggest that the name beet Western yellows be recognized and the name radish yellows dropped. This naming would be in accordance with the usage adopted by the Review of Applied Mycology in applying common names to virus diseases (1).

Studies in Europe and in the United States (2, 5, 6) have indicated that the sugar beet yellows virus in replicated field tests can cause reductions in sugar beet root yields of approximately 13-50%, depending on vigor of the plants, virulence of the virus isolate used, and date of infection.

Since it was known that the Western yellows virus, apparently unrelated to the beet yellows virus, was widespread in California sugar beet plantings in association with the beet yellows virus, it became of interest to determine more precisely 1) the relative incidence of the 2 viruses in such plantings, and 2) the damage inflicted in replicated field tests on the sugar yield of beets by the Western yellows virus alone and in combination with the beet yellows virus. The results of these studies are reported here.

RESULTS.—*Recovery tests from field sugar beets.*—Transmission tests from yellowed sugar beets in the Salinas and San Joaquin valleys of California and from the Medford and Salem areas of Oregon were conducted to determine the relative incidences of the beet and Western yellows viruses in these areas. The viruses were recovered by feeding nonviruliferous green peach aphids [*Myzus persicae* (Sulz.)] in the greenhouse on randomly collected field plants showing yellowing symptoms. The insects were allowed to feed on the plants for about 24 hours and then were transferred to healthy indicator seedlings for about 48 hours. The technique was essentially the same as described previously (3) and included the use of *Chenopodium amaranticolor* Coste & Reyn., *C. capitatum* (L.) Aschers, *Capsella bursa-pastoris* (L.) Medic., *Malva parviflora* L., and *Beta vulgaris* L., as test plants.

The results of these indexings (Table 1) indicated a high incidence of Western yellows virus in sugar beet plantings. In all areas tested, it was more prevalent than the beet yellows virus. In seed fields of Oregon, Western yellows virus was recovered from all 56 plants tested whereas beet yellows virus was recovered from only 1.

TABLE 1. Results of virus recovery tests from field sugar beet plants showing foliage yellowing, 1958-59

Area	No. of plants tested	No. of plants from which viruses were recovered		
		Western yellows virus only	Beet yellows virus only	Western and beet yellows viruses
San Joaquin Valley, Calif.—1958	40	25	0	3
Salinas Valley, Calif.—1958	20	15	2	3
San Joaquin Valley, Calif.—1959	107	38	21	36
Salinas Valley, Calif.—1959	152	55	25	37
Santa Clara Valley, Calif.—1959	24	15	0	1
Oregon Seed Fields—1959	56	55	0	1
Totals	399	203	48	81

Although relatively few plants were sampled in these tests, the relative distribution of the 2 viruses apparently has been obtained.

Field inoculation tests.—To determine the effects of the Western yellows virus alone and in combination with beet yellows virus on sugar yield, replicated field plots were established in a ⅓-acre field of U.S. 75 sugar beets planted January 10, 1959, near Salinas, Calif. Plots were 30 ft long and 3 rows wide and were arranged in 4 Latin Squares. The 4 treatments consisted of 1) uninoculated (control) plots, 2) plots inoculated with a severe isolate of the Western yellows virus, 3) plots inoculated with a severe isolate of the beet yellows virus, and 4) plots inoculated with both the Western and beet yellows isolates. Plants were inoculated during the week of April 1 by the method described by Bennett, *et al.* (2), in which leaf pieces, each with about 10 aphids, were removed from source plants and placed on plants being inoculated. Within 48 hours after inoculation and at approximately 2-week intervals thereafter until July 1, plots were sprayed with Systox at 1 pint/acre. The beets were harvested August 27. Yields and sucrose percentages were determined for each plot.

Percentages of infection obtained were approximately 84% in the Western yellows plots and 92% in the beet yellows plots. After inoculation of the other plots, the control plots had less than 1% infection. Aphid populations were low during early summer but tended to increase during the latter part of the season. By May 6, plots could be distinguished readily on the basis of color differences and remained that way until the latter part of July, when virus spread made distinctions more difficult. On the basis of intensity of yellowing, plants inoculated with the combination of Western and beet yellows viruses were the most severely affected. Plants in the beet and Western yellows plots followed, in that order.

TABLE 2. Effects of Western and beet yellows viruses on yield and sucrose content of sugar beet in tests at Salinas, Calif., 1959

Virus used as inoculum	Sucrose (%)	Yield of beets/A (T)	Gross sugar/A (lb)
None (control)	15.42[a]	37.64[a]	11,603[a]
Beet yellows virus	14.91[a b]	31.38[b]	9,358[b]
Western yellows virus	14.57[b]	32.16[b]	9,374[b]
Beet yellows virus + Western yellows virus	14.48[b]	26.13[c]	7,562[c]

[a,b,c] For a given trait, 2 means having any superscripts in common are not significantly different from each other at the 1% level.

Analysis of the sucrose-content data indicated that inoculation with both the Western and beet yellows viruses resulted in a significant lowering of sucrose percentage, compared with uninoculated sugar beets (Table 2). The reduction with beet yellows, however, was not significant at the 1% level. The interaction between the viruses, in regard to sucrose content, was not significant.

Analysis of the yield data indicated a highly significant effect resulting from inoculation with both beet and Western yellows viruses, but no interaction between the 2 types of yellows. The beet and Western yellows viruses caused yield reductions of 16.6 and 14.6%, respectively; whereas the combination of viruses caused a 30.6% reduction. The Duncan (4) comparison indicated that all yellows-inoculated plots yielded significantly less than the control. There was no significant difference between the yield effects of these isolates of the Western and beet yellows viruses, but the combination produced a highly significant reduction in yield, compared with effects of either virus alone.

Analysis of gross sugar data showed a highly significant reduction in sugar following inoculation with both Western and beet yellows viruses. A reduction of approximately 19% was recorded for both viruses, whereas the combination caused a reduction of 34.8%.

DISCUSSION.—The indexing of nearly 400 sugar beets from California and Oregon indicated a widespread occurrence of beet Western yellows virus. Although the number of plants sampled was relatively small, indexings have shown a high incidence of Western yellows virus in yellowed sugar beets, either alone or accompanied by beet yellows virus. In all areas tested, the Western yellows virus seemed to be more prevalent than the beet yellows virus.

In these tests, the degree of foliage yellowing in fields of sugar beets with mixed infections of the beet and Western yellows viruses was of little aid in determining the damage caused by them.

With its widespread distribution and prevalence in California and Oregon beet-growing areas and ability to cause serious yield reduction, beet Western yellows is a major sugar beet disease. Since resistance of plants to a virus does not, in general, give resistance to other viruses, it is important to have a breeding program include studies of resistance to the Western yellows virus as well as to the beet yellows virus.

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RELATION OF SOME EDAPHIC FACTORS TO AGROBACTERIUM TUMEFACIENS

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SUMMARY

The numbers of viable crown gall bacteria in soil were determined by a standardized plate count technique and pathogenicity test. An increase in numbers was observed in steam-sterilized soil under laboratory conditions. A more gradual decrease in numbers occurred in steam-sterilized soils than in unsterilized soils. The decline was greater and more rapid for coarser textured soils. A very gradual decrease in the number of crown gall organisms occurred at 2.5°C; whereas, a marked decrease in unsterilized soil occurred at 34°C. There was a direct relationship between numbers of *Agrobacterium tumefaciens* and soil moisture content of unsterilized soil contained in pots; an inverse relationship was observed under laboratory conditions. The crown gall bacterial population was greater at 0-4 in. than 8-12 in. below the surface of an unsterilized soil 159 days after the infestation of the soil. There was no appreciable difference in the numbers recorded between 0 and 23 in. in a chemically sterilized soil at 150 days. There was a direct relationship between the numbers of crown gall bacteria and the pH of soil to which sulfur had been applied.

The ability of *Agrobacterium tumefaciens* (Smith & Townsend) Conn to persist in soils has become increasingly important to growers and nurserymen. Many specific inquiries have been made about the effect of soil type, irrigation, and crop rotation, on the persistence of the crown gall organism in the soil.

Various reports state that the bacteria can remain viable in fallow soil for 127 (10), 154 (9), 186 (16), 420 (12), 422 (2), 732 (6), and 736 (13) days. Patel (13) readily recovered the bacterium from sterilized soil maintained in the laboratory after 736 days, less readily from unsterilized soil kept under the same conditions. The presence of pathogenic bacteria also was demonstrated for a longer period in sterilized soil than unsterilized soil by Gorlenko, *et al.* (5). Banfield (3) determined that the organism could overwinter under field conditions in Wisconsin and Patel (12) found that the bacteria were capable of overwintering under field conditions for 90 and 156 days in Iowa.

Observations have been made of the soil conditions and types conducive to the longevity of the organism. Reddick and Stewart (16) observed that the bacteria seemed to persist longer in moist than in dry field soil. Banfield (3) and Siegler (20) reported that a greater amount of infection occurred when susceptible hosts were growing in alkaline soils containing the pathogen compared with acid soils. Gorlenko, *et al.* (5) stated that soil reaction is an important factor in the loss of pathogenicity. Patel (12), Banfield (3), and Niemeyer (10) have indicated that a well-drained sandy loam soil favored longevity of the organism more than a heavy soil, especially if the latter were slightly acid. Patel (12) suggested that the sandy loam soil type supports fewer competing organisms and thus favors survival of *A. tumefaciens*.

MATERIALS AND METHODS.—Three isolations of *A. tumefaciens* were made and maintained in culture for all experiments. The original isolations were made from subterranean galls on almond, peach, and tomato plants. The soils for these tests were collected in several agricultural areas of California where a history of crown gall has been reported. The pH of the various soils was determined (1) at the time of collection and during the course of the experiments.

Bacteria were grown in 2-liter flasks containing potato-dextrose-peptone (PDP) broth (15) and incubated at room temperature (24-26°C) for 48-72 hours. Cells were separated by high speed centrifugation and washed 3 times in sterile distilled water. The soils were infested by uniformly atomizing an aqueous suspension of the crown gall organism on a thin layer of soil which was thoroughly mixed. The amount of inoculum introduced for each g of soil was comparable to that of soil surrounding galls of naturally infected almond and pear trees. Steam-sterilized, 8-in. pots were filled with the infested soil and placed on a thoroughly disinfested outdoor bench. A constant soil moisture content was maintained by subirrigation. This was done by introducing each day a determined amount of water through a glass tube containing glass wool inserted to 3.5 in. below the surface.

Soil for laboratory tests was placed in cotton-plugged Erlenmeyer flasks. The 1,000-, 500-, and 400-ml flasks contained 200, 100, and 75 g of soil, respectively. A drawn-out pipette was used to inoculate flasks with the appropriate amount of inoculum. Soil moisture content was maintained by weighing the flasks and adding sterile distilled water. Aseptic procedures were followed for the sterilized series of flasks.

The numbers of *A. tumefaciens* were determined by a standardized plate count technique and pathogenicity test. A disinfested cork borer was used to remove soil samples from test soils in pots. Samples for the counts at various depths were taken with a soil tube. Two samples were obtained for each count. A portion of each sample was used to determine the percentage of moisture (Pw) based on dry soil (8). Weight of soil samples varied between 35 and 40 g. Each sample was placed in 100 ml sterile distilled water in 400-ml Erlenmeyer flasks and agitated on a shaking machine for 1 hour. Ten ml of the soil suspension were immediately poured into a 90-ml water blank and shaken for 30 minutes on the shaker. Nine-ml water blanks were employed for the remaining serial dilutions of the suspension. One-ml amounts of the dilutions were placed in petri dishes and Patel's medium (11) was poured into the dishes and mixed by swirling the plates. One ml of 1:1,000 (wt/vol) Actidione (85-100% cycloheximide) was thoroughly mixed with 10 ml of the medium immediately before pouring into the plate. All plates were incubated at room temperature (24-26°C).

The plates were examined with a dissecting microscope following an incubation of 7, 14, and 21 days. A total count was made of all bacterial colonies, and those suspected of being *A. tumefaciens* were transferred to PDP agar slants. The maximum number of colonies transferred from a given plate was 25. A randomized sample of colonies typical of *A. tumefaciens* was taken from plates containing more than 25 suspected colonies. The slants were incubated at 28°C for 48 hours. Cells were removed from the slant with a sharp needle, and inoculated into tomato and sunflower plants by gentle punctures. The tomato plants were inoculated uniformly on the stem in the area between the cotyledons and the 1st leaves and the sunflower plants were inoculated between the 2nd and 3rd leaves. The plants were kept in the greenhouse at 60-70°F and were separated to prevent contamination by splashing water. Observations were made for the presence of galls 15-20 days after inoculation. The plants without galls were periodically inspected for 60 days.

The accuracy of the above method was ascertained by adding standard amounts of a crown gall bacterial suspension to various amounts of a suspension prepared from the soils used in these studies. An accuracy of approximately 90% or greater could be obtained when there were 1,500 or more crown gall bacteria/g of soil and the total number of colonies/plate was about 300 or less. Using the χ^2 and probability values for each dilution, the method of soil infestation appeared satisfactory because there was a uniform distribution of the crown gall bacteria in the artificially infested soil. The uniformity of infestation in the sandy soils was not as striking as in other soil types. The plate counts have been reported in the results as numbers of viable crown gall cells/g of soil (dry weight).

EXPERIMENTS AND RESULTS.—*Effect of steam sterilization.*—The flasks used for the laboratory experiments contained soil composed of 4 parts greenhouse loam and 1 part washed river sand. One series of flasks was steam-sterilized for 4 hours at 15 lb/in.^{2}; a 2nd series was not sterilized. A 3rd series of flasks was unplugged 8 days after sterilization and the soil placed in sterile petri dishes. The plates were placed outside in the open for 24 hours with the covers removed. The soil in each dish was then returned to its respective flask and the plug replaced. The latter series is referred to as "sterilized plus air contamination." The soil moisture content (Pw) was maintained at 11-14%. The flasks were kept at room temperature (24-26°C). The numbers of *A. tumefaciens* could not be satisfactorily determined after 90 days for the unsterilized series because of overcrowding of the dilution plates with other organisms.

Sterilized and unsterilized Yolo clay loam and Hanford loamy sand were used for pot experiments. The moisture content of the clay loam was kept at 50-55% of normal moisture capacity (18), whereas that of the loamy sand varied 55-60%. These soil moisture content values approximated those found at 4 in. below the soil surface when the soils were collected in the field.

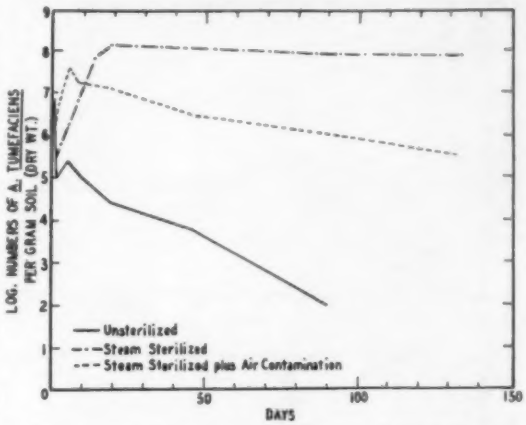

Fig. 1. Effects of steam sterilization and air contamination on *Agrobacterium tumefaciens* in a soil mixture composed of 4 parts greenhouse loam and 1 part washed sand contained in flasks kept at room temperature (24-26°C).

A more marked reduction in numbers of viable crown gall bacteria occurred in the unsterilized soils than in the steam-sterilized soils (Fig. 1, 2). This may be attributed to the combined effects of a lack of competitive microorganisms and a growth stimulation due to steam sterilization. The reduction due to air contamination of steam-sterilized soil (Fig. 1) strongly suggests the importance of the antagonism of other microorganisms on the persistence of the crown gall pathogen in soils. These results support the observations of Patel (12, 13).

The decrease in numbers in all soil series during the 1st 48-72-hour period was followed by an increase. This may have been due to the manipulation of the soil and the adding of moisture at the time of inoculation. Increase in total numbers of soil bacteria following moistening of air-dried soil has been reported by Rahn (14). He also noted a greater increase in loamy soils than in sandy soils. There is a slight reflection of this effect in the curves of Fig. 2, being more pronounced in the steam-sterilized soils. The curves of Waksman and Starkey (23) for the total numbers of bacteria in undisturbed potted soil under optimum conditions are similar to those obtained for *A. tumefaciens*, *i.e.*, an initial rapid increase followed by a decrease which became very slight after a period of about 200 days.

Effect of soil texture.—Some effects of soil texture are reflected in the crown gall population curves presented in Fig. 2. There was a more rapid reduction in the loamy sand soil than in the clay loam soil, particularly in the sterilized soils of each type.

A laboratory experiment was conducted by varying the amount of washed sand mixed with Yolo fine sandy

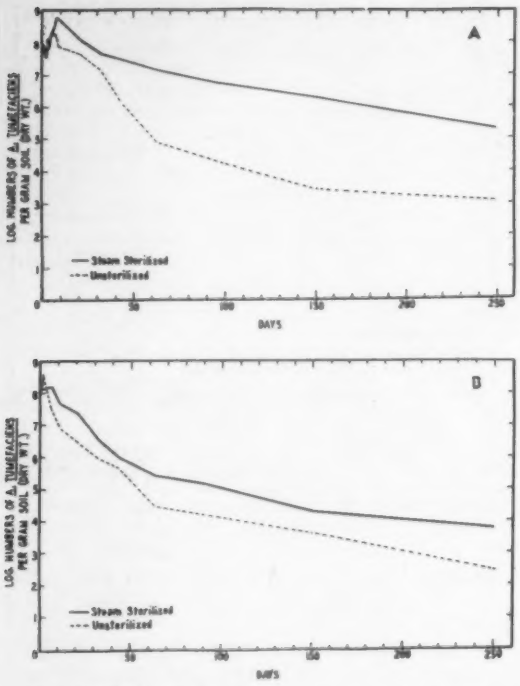

Fig. 2. Effect of steam sterilization on *Agrobacterium tumefaciens* in **A)** Yolo clay loam and **B)** Hanford loamy sand soil contained in pots kept out-of-doors.

loam soil. Yolo fine sandy loam soil was selected because it was easier to handle in flasks, the uniformity was good, and the amount of undecomposed organic matter was negligible. The artificially infested steam-sterilized and unsterilized soils were held at room temperature. The soil moisture content was maintained at 11-14%. Determinations of the number of *A. tumefaciens* were made 31, 61, 93, and 108 days after inoculation. There was no appreciable difference in the population curves for the steam-sterilized soil (Fig. 3-A). There was a more pronounced decrease in the unsterilized soil possessing a greater amount of sand after 31 days (Fig. 3-B).

Effect of soil moisture.—The artificially infested soil used for the laboratory experiments was composed of 4 parts Yolo fine sandy loam soil and 1 part washed sand. The flasks were held at room temperature.

Yolo fine sandy loam soil also was selected for the pot experiment. Determinations of the numbers of crown gall bacteria were made 1 day after artificial infestation. After the 2nd day, appropriate quantities of water were periodically added to each series to maintain soil moisture contents within desired limits.

There was a direct relationship between numbers of *A. tumefaciens* and the soil moisture content of steam-sterilized soil in laboratory tests (Fig. 4-A). The numbers of *A. tumefaciens* for 4.0-5.5% soil moisture were significantly lower (5% level) than those for the 2 highest moisture content values at both 74 and 95 days. A similar relationship occurred in the unsterilized soil contained in pots, except for series 2 at 105 days (Table 1). An inverse relationship was recorded for the unsterilized soil mixture in laboratory tests (Fig. 4-B). The latter effect may be attributed to an increase of other microorganisms resulting in a reduction in the population of the crown gall organism. The total bacterial counts did not necessarily reflect such an effect because the negative correlation coefficient value for the total bacterial population and *A. tumefaciens* was not significant; the pot experiment also did not demonstrate such an effect.

Soil microbiologists generally have found an increase in the total bacterial numbers accompanying an increase in soil moisture below the saturation point. The fact that the crown gall organism seems to survive longer in a moist field than in a dry soil has been reported (16).

Effect of temperature.—Steam-sterilized mixtures of 4 parts Yolo fine sandy loam and 1 part washed sand

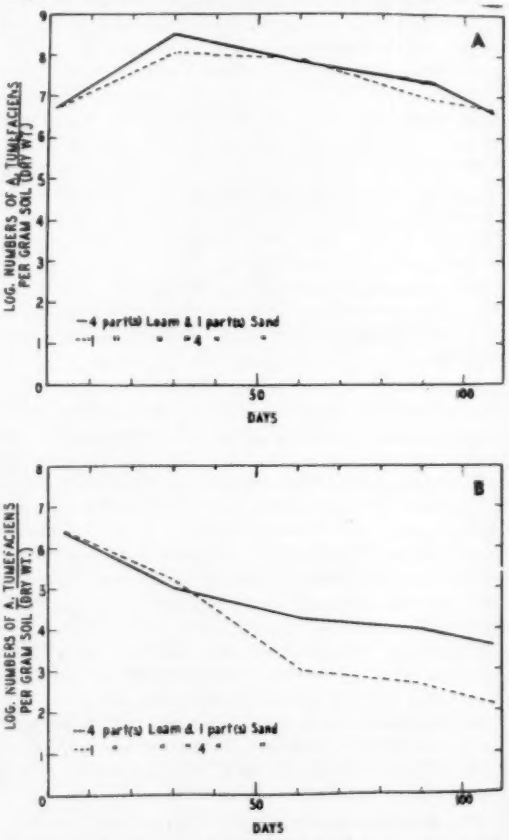
A
LOG. NUMBERS OF A. TUMEFACIENS PER GRAM SOIL (DRY WT.)
—4 part(s) Loam & 1 part(s) Sand
--1 " " " 4 " "
DAYS
B
LOG. NUMBERS OF A. TUMEFACIENS PER GRAM SOIL (DRY WT.)
—4 part(s) Loam & 1 part(s) Sand
--1 " " " 4 " "
DAYS

Fig. 3. Effect of soil texture on *Agrobacterium tumefaciens* in **A)** steam-sterilized and **B)** unsterilized soil mixtures. Yolo fine sandy loam and washed sand used to prepare the 2 mixtures.

were artificially infested with *A. tumefaciens* in flask containers. A mixture of 4 parts greenhouse loam and 1 part washed sand was used for the unsterilized flasks. Soil moisture content was maintained at 11-14%. Six flasks of each series were kept at each of 3

TABLE 1. Effect of soil moisture on the numbers of *Agrobacterium tumefaciens* in unsterilized Yolo fine sandy loam soil

Series	Days after soil infestation	Pw (dry weight) at time of sampling	No. of *A. tumefaciens*/g of soil (dry weight)
1	1	15.3	11,900,000
2	1	16.2	9,600,000
3	1	16.6	10,100,000
1	105	29.3	154,000
2	105	16.9	230,000
3	105	12.2	64,000
1	204	28.9	42,000
2	204	17.0	28,000
3	204	13.0	10,000

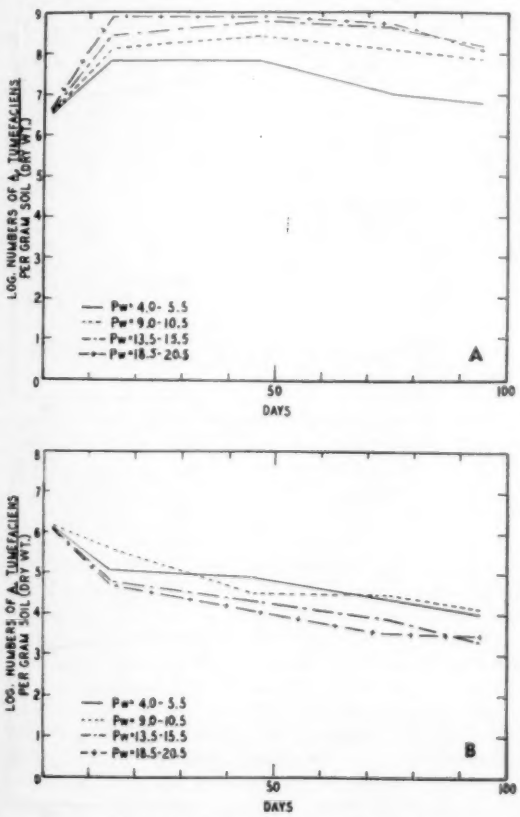

Fig. 4. Effect of soil moisture on *Agrobacterium tumefaciens* in **A)** steam-sterilized and **B)** unsterilized soil mixture composed of 4 parts Yolo sandy loam soil and 1 part washed sand contained in flasks kept at room temperature (24-26°C). Pw = percentage moisture based on dry soil.

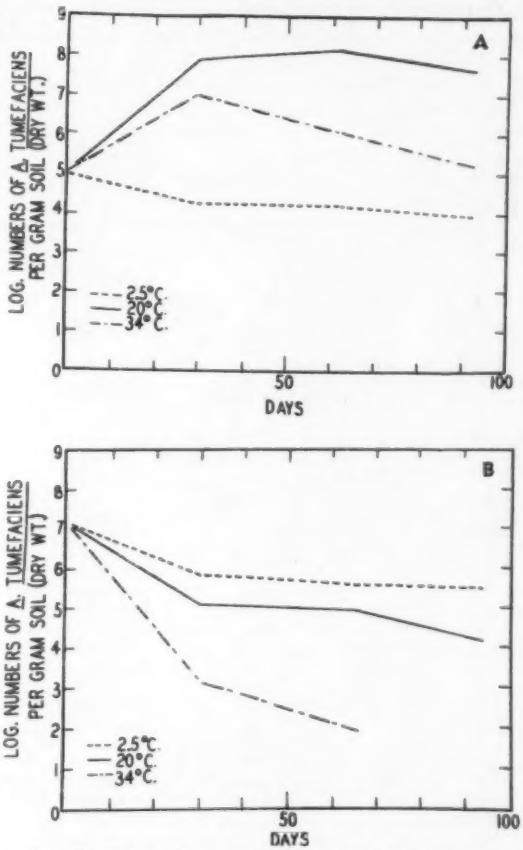

Fig. 5. Effect of temperature on *Agrobacterium tumefaciens* in **A)** steam-sterilized soil mixture composed of 4 parts Yolo fine sandy loam soil and 1 part washed sand and **B)** unsterilized soil mixture composed of 4 parts greenhouse loam soil and 1 part washed sand.

temperatures, 2.5°, 20.0°, and 34.0°C. A reading of the unsterilized soil at 34°C could not be made 90 days after inoculation due to the reduced number of crown gall organisms and consequent overcrowding of the dilution plates with other organisms. For the same reason, the value recorded for 62 days at this temperature is not as accurate as the others.

The results (Fig. 5) show that the longevity of the crown gall bacterium at 20°C in the steam-sterilized and unsterilized soils was comparable to that recorded in Fig. 1, although there was a temperature difference of approximately 5°C.

There was little difference between the steam-sterilized and unsterilized soils at 2.5°C. The recovery of the bacterium from infested field soils subjected to the low winter temperatures of Wisconsin and Iowa has been reported by Banfield (2) and Patel (13), respectively.

The most striking difference was observed at 34°C. Although an initial increase in numbers of the patho-

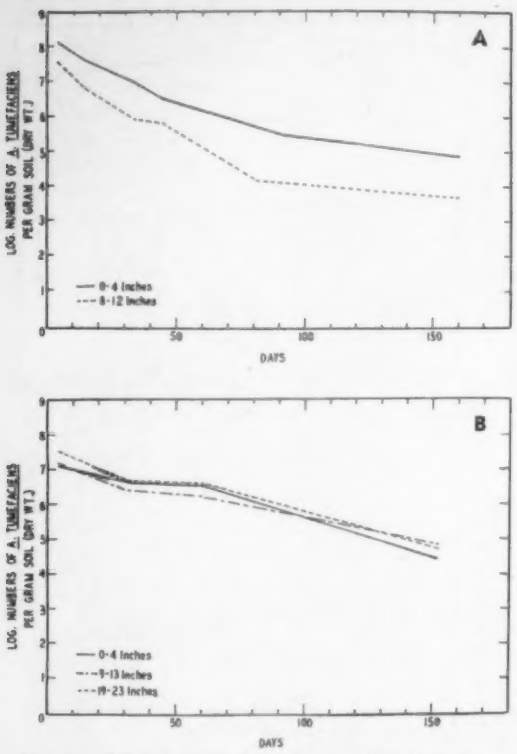

Fig. 6. Effect of soil depth on *Agrobacterium tumefaciens* in **A)** unsterilized and **B)** chemically sterilized greenhouse loam soil held in galvanized containers kept out-of-doors.

gen in steam-sterilized soil was followed by a decrease in the population, there was a very rapid reduction in the unsterilized soil. The rapid decline at 34°C in the unsterilized soil series possibly was more drastic under these laboratory conditions than in the field, because the soil more often reached the minimum 11% moisture content than did the soil of the other series. The high soil temperature coupled with more rapid drying probably was a primary factor in the decrease of the numbers of crown gall bacteria. Riker (17) placed inoculated tomato stems in soils held at various temperatures. He observed that gall development was markedly reduced at 30° and 34°C, compared with 22° and 26°C.

Effect of soil depth.—Artificially infested, unsterilized greenhouse sandy loam soil was placed in a 5-gal galvanized disinfested container. The moisture content of the top 4 in. of soil was maintained at 50-55% of normal moisture capacity. At 8-12 in. below the surface, the moisture content increased from 50% at the beginning to 71% at the termination of the experiment.

Chloropicrin-fumigated greenhouse loam soil was artificially infested and placed in a 10-gal disinfested galvanized container. The respective soil moisture

TABLE 2. Effect of pH on the numbers of *Agrobacterium tumefaciens* in Yolo clay loam soil

Treatment		Days after treatment	pH	No. of *A. tumefaciens*/g of soil (dry weight)
Steam-sterilized	None	0	6.9	175,000,000
		146	....	....
		220	6.6	89,500
	Sulfur	0	6.9	56,500,000
		146	4.9	31,000
		220	4.6	5,500
Unsterilized	None	0	7.2	13,000,000
		90	....	193,000
		146	7.0	25,500
	Sulfur	0	7.2	10,000,000
		90	....	3,500
		146	4.2	200

contents at the beginning and the end of the experiments were 51 and 54%, 51 and 69%, and 51 and 64% of the normal moisture capacity.

In the unsterilized sandy loam soil (Fig. 6-A), there was a decrease in population with an increase in depth. There was no noticeable decrease with depth in the chemically treated soil (Fig. 6-B), although slightly higher counts of the bacterium were recorded 150 days after inoculation at the greater depths. The higher soil moisture content may have been a favorable influence on the population at the greater depths as observed in preceding experiments.

Effect of pH.—Micronized elemental sulfur was mixed with steam-sterilized and unsterilized Yolo clay loam soil at the rate of 0.5% of total dry weight of soil. Sulfur was used because it has reduced disease incidence (7, 19, 22). The soil pH was determined prior to the addition of the sulfur and at the termination of the experiment.

The reduction in pH (Table 2) apparently caused a striking reduction in population of crown gall bacteria. A population reading was not obtained at 220 days for the treated unsterilized soil, and the reading for 146 days is not as accurate because the numbers of bacteria were extremely low. The rapid decrease in numbers in the unsterilized soil probably can be attributed in part to the rapid reduction in the soil pH due to the presence of a larger number of normal soil microorganisms, particularly the specific groups of sulfur bacteria, some of which may have been killed during the sterilization process. It is not assumed that factors, other than soil acidity, did not have an influence on the crown gall bacterium. The various products formed during the oxidation and reduction of elementary sulfur and their action on the crown gall bacterium cannot be discounted in the total effect.

DISCUSSION.—Some edaphic factors generally influencing soil microorganisms have been included in these studies. The procedure devised for determining the effect of various factors on numbers of crown gall bacteria was more precise than any other method tried. Although the experiments used for these longevity

studies were designed to limit the many influencing factors as much as possible, laboratory experiments were more easily controlled than those conducted out-of-doors in pots. Consequently, pot experiments were conducted so that the techniques and conditions were as uniform as possible.

The gradual decrease in numbers of *A. tumefaciens* at a low temperature (2.5°C) and the rapid decrease at a high temperature (34°C) are in accordance with the results of pure culture (21) and overwintering studies (3, 12). The outdoor and room temperatures which prevailed during the course of the experiments were found to cause a minimum of interference in the survival of the pathogen in the soil. The sampling of the soil in pots to a depth of 4.25 in. also has been shown to provide characteristic crown gall bacterial population determinations for the condition under which these experiments were conducted.

The ability of *A. tumefaciens* to survive for long periods in sterilized and unsterilized soil without the presence of an actively growing host has again been demonstrated. The bacterium apparently is capable of multiplying in sterilized soil without the loss of pathogenicity, but multiplication in unsterilized soil is not adequately demonstrated. These studies did not attempt to ascertain the effect of the metabolic processes, both of the organism itself and surrounding microflora, neither of which can be discounted. The antagonism of other microorganisms was demonstrated to decrease the numbers of the crown gall bacteria in the soil (Fig. 1). The antagonistic effects may be attributed to 1 or several factors, such as staling, parasitism, antibiosis, or competition for space, nutrients, or other requirements. Although the results do not provide conclusive evidence that *A. tumefaciens* is able to maintain itself in the soil as a saprophyte, the bacterium must be considered as a successful soil invader (4).

The results of experiments reported herein indicate that a striking reduction of the pathogen in the soil can be accomplished by the addition of sulfur which reduces soil pH. This agrees with previous reports (3, 5, 7, 19, 20, 22) on the effect of sulfur and soil pH. It also can be assumed that a soil which is kept very warm and dry would be less satisfactory for the survival of *A. tumefaciens* than if it were cool, moist, and well drained. This again concurs with field and laboratory observations (3, 10, 12, 16).

The reports reviewed (3, 10, 12) have considered sandy type soil more favorable for longevity of the crown gall pathogen than other soil types. These reported conclusions have been based on the occurrence of the disease (3) or on results of inoculations by means of soil suspension (12), rather than upon comparative suitability of the soils for survival and multiplication. This fact may account for some of the discrepancy between the results of the present studies (Fig. 2, 3) and those reported by other workers. It is generally known that the addition of organic material to soil causes an increase in the numbers and influences the types of organisms. The increase in the microbial population undoubtedly would influence the effect of antagonism which has been demonstrated. Therefore, the effect of antagonism due to the organic matter content of the soils might be greater than the effect of the soil texture, and thus cause these differences of results. The laboratory results (Fig. 3) also may reflect a dilution of the organic material because little or none was added when washed sand was mixed with the soil. The dilution effect was not apparent in the sterilized soil (Fig. 3-A), but it may be coupled with the effect of antagonism by other microorganisms present in the unsterilized soil (Fig. 3-B).

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RELATIVE CONCENTRATION OF CUCUMBER MOSAIC VIRUS IN A RESISTANT AND A SUSCEPTIBLE CUCUMBER VARIETY

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SUMMARY

Partially purified extracts from leaves of varieties of cucumber, both resistant and susceptible to cucumber mosaic virus, were assayed for virus content at 4-day intervals beginning 4 or 6 days after inoculation. The purification process removed most of the inhibitor normally present in leaf extracts of healthy and infected tissue. The concentration of inhibitor was similar in resistant and susceptible cucumber extracts. There was a cyclical pattern of virus multiplication in each variety. The virus titer was usually much lower at any given interval in extracts from resistant plants than from susceptible plants. The resistance mechanism apparently suppresses, but does not exclude, systemic virus multiplication.

In another paper (6) we showed that resistance to cucumber mosaic virus (CMV) in certain varieties of cucumber was controlled by a single dominant gene. It had previously been shown (3) that in such resistant varieties the virus developed systemically but virus assay by the cowpea local-lesion method (2) was unsatisfactory because of interference by an inhibitor present in cucumber leaf tissue extract. The present paper deals with a continuation of this study in which a satisfactory assay was developed with partially purified virus extracts.

MATERIALS AND METHODS.—Two varieties used in the previous study (6) were chosen since they were very similar genetically except for the resistance gene allele. Wisconsin SR 6 is homozygous for susceptibility and Wisconsin SMR 15 is homozygous for resistance to mosaic. The virus strain and methods of maintenance of virus source plants and of inoculation have been described (6). Cucumber plants were grown throughout at 26-28°C and inoculations were made on cotyledons at the 1st leaf stage. When virus titer was being studied, the 3 youngest systemically infected leaves were collected from 70 or more plants for each assay. Following the method of Tomlinson, Shepherd, and Walker (5), leaves were macerated in 0.5 *M* potassium phosphate buffer (pH 7.5), containing 0.7% thioglycolic acid. The homogenate was filtered through a double layer of cheesecloth, the volume of the extract was measured, and butanol was added dropwise with constant stirring to give final concentration of 9%. After stirring for 30 minutes, the mixture was centrifuged 15 minutes at 5,000 rpm. The supernatant was filtered through glass wool and centrifuged for 2 hours at 30,000 rpm. The pellet was resuspended in 1.0-1.5 ml of 0.05 *M* buffer (pH 7.5) on a mechanical shaker over an 18-hour period and the original volume was restored with the same buffer. The purification procedure was carried out at 3°. The final preparation undiluted and diluted 1:5 in 0.05 *M* buffer showed a high virus titer when inoculated on cowpea, whereas inoculation with the supernatants from each centrifugation, as well as with the original homogenate, showed only 1 lesion or less/10 leaves inoculated. This showed that a large portion of the inhibitor had been removed in the purification.

RESULTS.—*Relative amounts of inhibitor in resistant and susceptible leaf tissue.*—An extract in 0.05 *M* buffer of infected cucumber corolla tissue containing a high titer of virus was brought to a 1:10 dilution in each of the following dilutions of healthy SR 6 leaf tissue extract: 1:100, 1:200, 1:400, 1:600, and 1:1,000. Each diluted extract was applied to 1 primary leaf of each of 10 cowpea plants; the opposite leaf of each plant was inoculated with the original corolla extract diluted 1:10 with 0.05 *M* buffer. It has been shown (3) that corolla tissue contains very little inhibitor. The degree

TABLE 1. Concentration of cucumber mosaic virus in extracts of young leaves of susceptible (SR 6) and resistant (SMR 15) cucumber at various intervals after inoculation as shown by local lesion assay on cowpea; experiment conducted in June and July

Days after inoculation	Avg. no. of lesions/cowpea leaf			
	Undiluted extract		Extract diluted 1:5	
	SR 6	SMR 15	SR 6	SMR 15
6	843	492	745	240
10	5	2	0	0
14	10	1	0	0
18	29	74	1	10
22	220	134	73	27
26	95	131	71	33
30	67	15	9	1
34	22	1	0	0

TABLE 2. Concentration of cucumber mosaic virus in extracts of young leaves of susceptible (SR 6) and resistant (SMR 15) cucumber at various intervals after inoculation as shown by local lesion assay on cowpea; experiment conducted in September and October

Days after inoculation	Avg. no. of lesions/cowpea leaf			
	Undiluted extract		Extract diluted 1:5	
	SR 6	SMR 15	SR 6	SMR 15
4	240	22	7	1
8	588	101	46	14
12	710	39	113	2
16	6	1	0	0
20	23	1	1	0
24	53	34	5	4
28	110	44	11	5
32	49	7	7	1

of inhibition of the virus was expressed as "percentage of inhibition." This was derived by first determining the difference between the average number of lesions on control leaves and the average number of lesions on leaves inoculated with a given control sap/leaf sap dilution. This latter value was divided by the average number of lesions/control leaf and the quotient was multiplied by 100. Inhibition for 1:100 dilution of healthy cucumber extract was 60%; for 1:200 dilution, 33%; for 1:400 dilution, 20%; for 1:600 dilution, 15%; for 1:1,000 dilution, 7%. This showed that a considerable amount of inhibitor remained in healthy leaf extract diluted 1:100.

In order to determine whether or not the inhibitor concentration differed in SMR 15 from that in SR 6, the extracts from healthy plants were compared by inoculating opposite leaves of 20 cowpea plants with infected corolla tissue extract to which leaf extract from the respective varieties was added; final dilution of the leaf extract was 1:100. The average number of lesions/cowpea leaf inoculated with the SR 6 extract was 256 and that for SMR 15 was 276. This indicated no appreciable difference in the amount of inhibitor in leaf tissue of the resistant and the susceptible variety.

Virus titer in resistant and susceptible plants.—The 1st experiment was conducted during June and July. The 1st sign of disease appeared on the fourth day after inoculation as epinasty of the 1st leaf in the susceptible variety. This was followed promptly by mottle in both varieties; as previously described (6), the symptoms declined rapidly in the resistant variety. Leaves were collected for assay on the sixth day after inoculation and at 4-day intervals thereafter, up to and including the 34th day. After purification, extracts were restored to the original volume and used undiluted and at 1:5 dilution in 0.05 *M* buffer. The results (Table 1) show that the virus titer was highest at 6 days in each variety. The concentration dropped very abruptly at 10 days, began to rise again at 18 days, and reached a peak at 22 days. This was followed by a second decline.

A second experiment was conducted from September 3 to October 14 when day and night periods were about equal in length and growth of plants was somewhat slower. The 1st assay was made at 4 days. Table 2 shows a cyclical pattern in virus multiplication. In this case, the 1st peak was reached at 8 days in SMR 15 and at 12 days in SR 6. The second peak was reached in both varieties at 28 days. In both experiments the titer of the extract of resistant SMR 15 was consistently lower than the corresponding titer of susceptible SR 6.

DISCUSSION.—Cheo and Pound (1) showed a cyclical pattern of cucumber virus concentration in systemically infected leaves of spinach and tobacco. Tomlinson, Shepherd, and Walker (5) noted a similar pattern in inoculated leaves of tobacco. We reported a similar case in pumpkin (6). The results presented herewith show clearly that a cyclical pattern prevails in cucumber. In the susceptible variety, there was some variation in severity of mottle symptoms during the experimental periods, but there was no correlation between severity and virus titer. In the resistant variety, the same cyclical pattern of virus concentration occurred as in the susceptible variety. In both experiments, the peaks of virus titer were reached in the 2 varieties on the same day or within 4 days of each other. The chief difference between the 2 varieties was: the number of local lesions for the resistant variety generally was very much lower than the corresponding number for the susceptible variety. It would appear that the resistance mechanism in this case is one which restricts the virus multiplication sufficiently to minimize the detrimental effect upon the growth and productivity of the host.

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EFFECTS OF CONCENTRATION OF HOST NUTRIENTS ON PARASITISM OF PIPTOCEPHALIS XENOPHILA AND P. VIRGINIANA

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SUMMARY

The degree of parasitism of *Piptocephalis xenophila* on *Penicillium frequentans* growing on agar media varied with the source and concentration of nitrogen and the ratio of carbon to nitrogen. On a glucose-yeast extract medium, growth of the parasite on its host was correlated with high nitrogen and inversely related to concentration of sugar in the medium.

Piptocephalis virginiana, parasitizing cultures of *Mortierella pusilla*, appeared within 5 days on a liquid medium composed initially of 5 g glucose and 5 g yeast extract/liter. At that time the sugar supply was nearly exhausted. Maximum growth of the parasite was attained within 11 days. In cultures receiving frequent additions of glucose to maintain a high carbon concentration, the growth of the parasite was delayed indefinitely. When the concentrations of both glucose and yeast extract were maintained high by frequent additions, however, the parasite appeared within 9 days and reached its maximum growth within 15 days.

A direct relation was found between the concentration of nitrogen in the host medium and the amounts of soluble nitrogen in the mycelium of 2 host fungi.

Piptocephalis xenophila was highly parasitic on *Penicillium frequentans* in liquid media only after high concentrations of microelements were added. Manganese was the most effective element.

INTRODUCTION.—Studies of mycoparasites have, for the most part, been limited to host ranges and mode of parasitism. Physiological studies of the nutritional and environmental factors that modify the degree of parasitism have received little attention.

Piptocephalis spp. produce branched haustoria within the host hyphae and are known as obligate parasites, principally on species of the *Mucorales*. They have never been grown, except for limited mycelium and dwarf sporangiophores, in the absence of a living host (Berry and Barnett, 5). This is evidence that these parasites cannot absorb all of their required nutrients from the host medium, but must obtain 1 or more nutrients from the host mycelium. Additional results obtained in our laboratory (unpublished) have shown that *P. virginiana* was capable of parasitizing and making fair growth on mycelium of *Mortierella pusilla* that had been washed in distilled water and starved as long as 7 days.

Ayres (1), working with *Dispira cornuta* van Tiegh., early demonstrated that a medium rich in nitrogen favored the growth of the parasite on its hosts. Berry (4) found that the carbon source, nitrogen source, and the initial ratio of carbon to nitrogen in the medium affected the degree of parasitism of *P. virginiana* Leadbeater & Mercer on species of Mucorales. The morphology and mode of parasitism of *Piptocephalis* have been described (3, 6, 10, 11). Berry (4) failed to find, by use of paper chromatography, any specific amino acid in the host mycelium, required for parasite growth.

Increased growth of *Calcarisporium parasiticum* Barnett on *Physalospora* spp., resulting from increased concentrations of nitrogen in the medium, was reported by Barnett and Lilly (2). More recently, Shigo (13) described the effects of carbon and nitrogen sources and of additional microelements on parasitism of *Gonatobotryum fuscum* Sacc. on *Ceratocystis*, *Graphium*, and *Leptographium* spp.

The present paper reports briefly the results of portions of an extensive study of fungi parasitic on other fungi designed to learn more about the basic

TABLE 1. The degree of parasitism of *Piptocephalis xenophila* on *Penicillium frequentans* growing for 10 days on agar media differing in amount of glucose and source and amount of nitrogen: rating 0 (none)—4 (excellent)

Nitrogen source	g glucose : g nitrogen/liter			
	5:2.3	1:0.46	5:0.46	10:0.46
Ammonium nitrate	0	2	0	2
Ammonium tartrate	3	2	0	0
Asparagine	4	2	2	0
Casamino acids	3	2	0	0
Glutamic acid	0	2	4	0
Potassium nitrate	2	2	2	2
Urea	0	2	2	0
Yeast extract	4	2	1	0

principles of parasitism. It is the third in a series of papers dealing with *Piptocephalis* spp.

MATERIALS AND METHODS.—The fungi used in this study were selected from the West Virginia University culture collection. *P. virginiana* was collected near Morgantown, W. Va., in 1953, and *P. xenophila* Dobbs & English was collected near Asheville, N. C., by C. R. Berry in 1957.

A glucose-yeast extract medium was used in most of the experiments. Agar was added for petri dish cultures. Synthetic media used in some experiments were composed of D-glucose (variable amounts), nitrogen source (variable amounts); KH_2PO_4, 1.0 g; $MgSO_4.7\ H_2O$, 0.5 g; Fe^{+++}, 0.1 mg; Zn^{++}, 0.1 mg; Mn^{++}, 0.05 mg; Ca^{++}, 10 mg; distilled water, 1 liter. The initial pH of all media was adjusted to 6.0 before autoclaving at 121°C for 15 minutes.

Unless otherwise stated the liquid cultures contained 25 ml of medium in 250-ml Erlenmeyer flasks. Spores were used as inoculum. The cultures were incubated in a constant temperature room at 25°C. with 12 hours of fluorescent light daily. Other special methods are described later with the appropriate experiments. All data represent averages of duplicate cultures.

Since *Piptocephalis* spp. are obligate parasites causing no visible damage to their hosts, except a slight decrease in growth, the only feasible method of measuring the parasitic activity is by estimating the amount of growth of the parasite. This is believed to be an accurate measure of the degree of parasitism.

RESULTS.—*Effects of source and amounts of nitrogen.*—The degree of parasitism of *Piptocephalis xenophila* on *Penicillium frequentans* was determined on 32 agar media varying in amount of glucose, and amount and source of nitrogen. Since a medium containing 5 g glucose and 5 g yeast extract (designated as a 5:5 medium) gave excellent parasitism, and a 5:1 medium was poor for growth of the parasite, these 2 media were used as standards for comparison. One g of yeast extract contains approximately 0.46 g nitrogen. Other nitrogen sources were added in amounts to give equivalent amounts of nitrogen. The amount of glucose varied 1-10 g/liter. The degree of parasitism, judged by the amount of growth of *P. xenophila*, was observed daily and rated 0-4. The results after 10 days are presented in Table 1.

In general, the media high in glucose and low in nitrogen were poor for parasitism, although they allowed excellent growth of the host. Parasitism was intermediate on all media that were low in both glucose and nitrogen. An intermediate amount of glucose with high nitrogen resulted in the best parasitism on media containing ammonium tartrate, asparagine, casamino acids, and yeast extract. The results on glutamic acid, ammonium nitrate, and urea did not fit into this pattern and cannot be explained on the basis of our present knowledge.

Yeast extract was one of the best nitrogen sources and the degree of parasitism appeared related to the concentration of yeast extract, which contains not only a mixture of amino acids but also furnishes the required metals, microelements, and vitamins.

Relation of parasitism to concentrations of glucose and yeast extract in the medium.—The effects of nutrient concentration were tested further using liquid media. Since *Piptocephalis xenophila* made little or no growth on *Penicillium frequentans* in a wide variety of liquid media, it was decided to test this hypothesis using *Piptocephalis virginiana* growing on *M. pusilla*.

The 1st portion of this experiment was carried out in a medium containing 5 g of glucose and 5 g of yeast extract/liter. Fifteen-ml portions of this medium in 250-ml flasks were inoculated with a mixture of host and parasite spores. The cultures then were divided into 3 lots, the 1st lot receiving no added nutrients during the growth of the mycelium. To the cultures of the 2nd lot, enough glucose was added at frequent intervals to restore the level of glucose to approximately the initial concentration. Under these conditions the supply of available nitrogen would soon become very low.

The residual glucose in sample cultures of each lot was determined daily using a modified Folin-Wu method; the calculated amount necessary to restore the concentration to the desired level then was added to the appropriate cultures. It was not possible by this method to maintain a uniform concentration throughout the incubation period, but it was always kept relatively high. To the 3rd lot of cultures, enough glucose and yeast extract in equal proportions by weight were added to maintain the level of glucose at approximately the original concentration. In these cultures there would be an increasingly higher ratio of yeast extract to glucose in the medium. The results of the 1st portion of the experiment are presented in Fig. 1.

In the cultures receiving no additions, the glucose supply was nearly exhausted at the end of 5 days. Growth of the parasite, judged by the presence of conidiophores, was first evident at that time and reached a maximum within 11 days. In the cultures which received frequent additions of glucose alone (300 mg over the 15-day period), the residual sugar was maintained at 40-110 mg/culture. There was no visible growth of the parasite at the end of 15 days. In the 3rd lot of cultures which received frequent

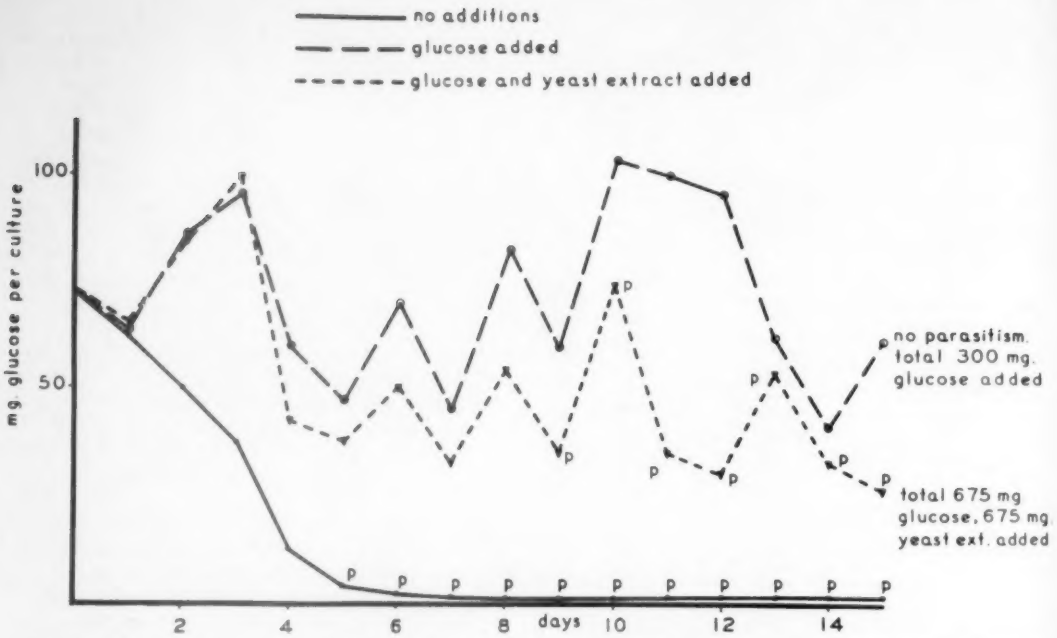

Fig. 1. The effects of frequent additions of glucose and equal amounts of glucose and yeast extract on the growth of *Piptocephalis virginiana* on *Mortierella pusilla* on liquid media initially containing 5 g glucose and 5 g yeast extract/liter. P = presence of the parasite.

additions of equal amounts, by weight, of glucose and yeast extract, the sugar concentration varied during incubation 23-95 mg/culture. The additions over the 15-day period totaled 675 mg each of glucose and yeast extract. The parasite appeared on the 9th day and reached maximum growth at 15 days.

The 2nd portion of the experiment was similar, except that 2 media were used, having concentrations of glucose and yeast extract of 10:5 and 25:5. These also were divided into 3 lots, 2 of which received frequent additions of nutrients to maintain high concentrations.

When the medium initially contained 10 g glucose /liter, there was no growth of the parasite after 15 days in cultures receiving added glucose only. Parasitism was delayed in cultures receiving both glucose and yeast extract and those receiving no added nutrients. When the initial medium contained 25 g glucose/liter, there was no visible growth of the parasite under any condition at the end of 15 days.

Briefly, these results show that relatively high concentrations of yeast extract favored the growth of the parasite, while high concentrations of glucose restricted the growth of *P. virginiana* on *M. pusilla*.

The relation between nitrogen content of the medium and soluble nitrogen content of host mycelium.—While it was assumed that a high concentration of nitrogen in the host medium resulted in an increase in the nitrogen available to the parasite, it could not be said with certainty that the parasite absorbed its nitrogen from the host cells or directly from the medium with which it was constantly in contact.

In order to determine whether a relationship existed between the amounts of carbon and nitrogen in the medium and the amount of soluble nitrogen in the host mycelium, *M. pusilla*, a susceptible host, was grown in 4 liquid glucose-yeast extract media containing the following amounts in g/liter: 5:1, 25:1, 5:5, 25:5. Cultures were harvested after 5, 7, and 9 days.

The soluble nitrogen was determined by adding 50 ml of cold 5% trichloroacetic acid to the mycelium from 2 cultures, allowing the extraction to continue in the cold with frequent stirring for 2 hours. This solution was filtered off and the mycelium washed twice with 5% trichloroacetic acid. The amount of nitrogen in the solution then was determined by the usual procedure using the micro-Kjeldahl apparatus (Table 2). Hereafter, in this paper the term "soluble nitrogen" is used to designate the nitrogen obtained in the way described above.

The mycelium of *M. pusilla* grown in a 5:5 glucose-yeast-extract medium contained slightly more than twice as much soluble nitrogen as did mycelium grown in the 5:1 medium. This was consistent in the 3 harvest periods. When the concentration of glucose was increased to 25 g/liter, however, the greater amount of yeast extract caused only a relatively slight increase in soluble nitrogen content of the mycelium. Media high in glucose resulted in low content of soluble nitrogen in the mycelium, presumably because of the

TABLE 2. The relation of amounts of glucose and yeast extract to amounts of TCA-soluble nitrogen in the mycelium of *Mortierella pusilla*; percentage of soluble nitrogen is based on dry weight of mycelium

Age of cultures (days)	Data/culture	Initial medium: g glucose : g yeast extract 5:1	25:1	5:5	25:5
5	g dry mycelium	61	156	115	154
	% TCA—nitrogen	0.73	0.48	1.50	0.64
	mg residual nitrogen	1.02	1.12	5.1	5.99
	mg residual sugar	57	543	15	506
	pH	5.9	4.4	4.6	4.0
7	g dry mycelium	82	185	138	256
	% TCA—nitrogen	0.61	0.34	1.46	0.40
	mg residual nitrogen	1.32	1.38	10.5	11.1
	mg residual sugar	13	394	5	339
	pH	4.1	7.2	4.6	4.1
9	mg dry mycelium	87	226	126	353
	% TCA—nitrogen	0.68	0.32	1.61	0.55
	mg residual nitrogen	1.38	1.08	5.92	4.62
	mg residual sugar	3	332	4	100
	pH	4.1	7.5	4.4	4.3

greater amounts of nitrogen tied up in protoplasm and cell wall material.

A similar but shorter experiment was carried out using *Penicillium frequentans*, host of *Piptocephalis xenophila*, and comparing the percentage of soluble nitrogen in the host mycelium with that of the host and parasite mycelia together (Table 3).

These data show that the presence of the actively growing parasite had little effect on the total soluble nitrogen within the mycelium of the host. At the end of the 3rd day, there was only slightly more soluble nitrogen in mycelium grown in 5 g/liter of yeast extract than in mycelium grown in medium containing 1 g/liter. During the period 7-12 days, however, the difference in soluble nitrogen was approximately 3-fold. Thus, in *Penicillium frequentans*, as in *M. pusilla*, there is a direct correlation between the amount of soluble nitrogen in the mycelium and the growth of *Piptocephalis* on its host.

Effects of increased concentration of microelements on parasitism.—During the course of the investigations, it was observed that the degree of parasitism of *Piptocephalis xenophila* on *Penicillium frequentans* was greater on glucose-yeast extract agar medium than on the same medium without agar, although the host grew equally well in the liquid medium. This effect may have been due to the additional microelements in the agar.

Liquid media, composed of 5 g glucose and 5 g yeast extract/liter and containing different amounts of microelements, were inoculated with a mixture of spores of host and parasite. After 8 days only slight growth of the parasite (rated as 1) occurred in the control cultures receiving no additional microelements, whereas excellent growth of the parasite (rated as 4) occurred in the cultures containing 0.25 mg Mn, 0.5 mg Fe, 0.5 mg Zn, and 5 mg Ca/liter. Four times these amounts of microelements caused no inhibitory effect. The degree of parasitism was unchanged after 12 days. When manganese was the only microelement added in concentrations of 0.05, 0.25, and 1.0 mg /liter, parasitism was rated 1, 3, 4, respectively. Similar increases in degree of parasitism resulted when the microelements were added to media containing potassium nitrate, asparagine, or phenylalanine as the nitrogen source. The added microelements did not, however, increase the degree of parasitism on a glutamic acid medium.

DISCUSSION.—The results presented above indicate that growth of the 2 *Piptocephalis* spp. on their host fungi is greatly affected by the concentrations of carbon and nitrogen in the medium on which the host is growing. Parasitism appears directly correlated with the concentration of available nitrogen in the medium and the percentage of soluble nitrogen in the host mycelium, and inversely related to the concentration of glucose in the medium. High concentration of available carbon probably leads to rapid depletion of nitrogen and results in nitrogen starvation, unfavorable for parasitic activities of *Piptocephalis* spp. Similar relationships were found for *Calcarisporium parasiticum*, a biotrophic parasite on *Physalospora* spp. and related fungi (2).

When the host medium contained 1 g of glucose and 1 g of yeast extract/liter, the growth of the parasite was fair (rated as 2) (Table 1). An increase in the original concentration of glucose in the same medium to 5 g/liter resulted in a decrease in parasitism, while 10 g of glucose prevented growth of the parasite for 10 days or more. Also, the growth of the parasite was completely inhibited for 15 days when the glucose concentration of the medium was maintained near 5 g/liter by frequent additions of this sugar (Fig. 1). This inhibition cannot be attributed to a direct effect of increased glucose, for no inhibition of the parasite was observed when the yeast extract was increased to

TABLE 3. The relation of amount of yeast extract in the medium to the amount of TCA-soluble nitrogen in the mycelium of the host (*Penicillium frequentans*) compared with that of the combined mycelia of host and parasite (*Piptocephalis xenophila*). Initial glucose concentration of all media was 5 g/liter

Days	Host mycelium alone: 1 g yeast extract mg mycelium	1 g yeast extract % nitrogen	1 g yeast extract mg mycelium	1 g yeast extract % nitrogen	Host-parasite mycelia: 5 g yeast extract mg mycelium	5 g yeast extract % nitrogen	5 g yeast extract mg mycelium	5 g yeast extract % nitrogen
3	86	0.94	113	1.00	53	0.92	102	1.09
7	114	0.97	117	2.24	80	0.86	137	2.35
10	88	0.71	149	2.91	80	0.70	117	2.39
12	84	0.66	138	1.89	70	0.75	96	2.26

5 g/liter. Furthermore, there is no evidence that glucose in a concentration of 5 g/liter will inhibit the growth of any fungus in a complete medium. The only logical conclusion is that the higher concentration of glucose affected the parasite through the living cells of the host.

Similarly, the greater degree of parasitism on some media in which the yeast extract was increased from 1 to 5 g/liter cannot be attributed entirely to a direct effect on the parasite, for no increase in growth of the parasite was observed when the initial concentration of glucose was 1 g/liter (unpublished data).

The role of microelements in parasitism is not clear, but there is sufficient evidence to warrant further investigation, both with fungi and with higher plants.

High nitrogen nutrition of some higher plants also has been correlated with increased susceptibility to certain fungi and bacteria (7, 8, 9, 14, 15). In only a few of these host-parasite relationships, however, has susceptibility been correlated with increased nitrogenous compounds within the host cells (7, 12, 15).

Thus, there is a striking similarity in the response of biotrophic parasites of fungi and those of higher plants to changes in host nutrition. This suggests that the basic metabolic activities underlying parasitism may be similar in many cases, and that mycoparasites may be used to some extent in studies of the principles of parasitism. Mycoparasites require relatively little time and space for their culture and the environment of the host, as well as its nutrition, can be controlled accurately, without the complicating process of photosynthesis that occurs in green plants.

Numerous parasites, illustrating different types of parasitism among fungi, are available for study. Such studies should supplement those with higher plants and should lead to a better understanding of the principles of parasitism.

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GERMINATION OF CHLAMYDOSPORES OF FUSARIUM SOLANI F. PHASEOLI IN UNSTERILIZED SOILS

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SUMMARY

Chlamydospores of *Fusarium solani* f. *phaseoli* formed in unsterilized soils fortified with the fungus did not germinate when the soils were saturated with water alone, but they did germinate in water-saturated soils 18 hours after being placed in contact with excised bean stems in petri dish moist chambers. The zone of chlamydospore germination occurred in the proximity of the host surface and was not observed to occur farther out, even after 1 week. Macroconidia, freshly added to soil, germi-

nated more rapidly and uniformly than did chlamydospores and were not influenced in their germination by the proximity of the host tissues. The observations on chlamydospores suggest that the fungus is usually passive in soil and that infection occurs chiefly through the chance encounter of the host with the pathogen.

INTRODUCTION.—The study of the behavior of soilborne plant pathogens has lagged behind that of pathogens attacking the aerial parts of plants, principally because of the lack of suitable techniques of observation. Recently, some of these techniques (4, 5, 6, 7) have enabled the behavior of *Fusarium solani* (Mart) Appel & Wr. f. *phaseoli* (Burk.) Snyd. & Hans. to be followed both in soil and on the host, in nature and in the laboratory.

This paper presents results of laboratory observations on germination and growth of chlamydospores and conidia of the fungus in unsterilized soil, leading up to penetration of the host.

MATERIALS AND METHODS.—Two soil types from the Salinas Valley were used: a sandy loam from a bean field and a loamy sand from a noncultivated area. Nutrient-medium-free macroconidial suspensions of a common, pathogenic, sporodochial clone of *F. solani* f. *phaseoli* were added to the 2 nonsterile soils which had been placed in 6 x 6 x 4-in. wooden boxes. The soils dried down gradually and remained undisturbed in the laboratory for 2 months at which time the fungus existed solely as chlamydospores. After this period and during the course of the experiments, the numbers of chlamydospores remained essentially unchanged with plate counts showing an average of 100,000 chlamydospores/g of soil.

Pinto bean seedlings grown in the greenhouse were excised when 6 in. tall, were surface sterilized, trimmed to 3-in. sections of stem, and placed in petri dish moist chambers following the method of Toussoun, *et al.* (7). Portions of soil to be tested were brought up to saturation and then placed on the stems as small lumps approximately 10 mm long and 4 mm high. Soil placed on glass rods served as controls.

The behavior of the chlamydospores was observed microscopically by staining soil that had been thinly spread over a slide and by staining epidermal strips from which the soil had been gently washed.

All experiments were done at room temperature, contained 6-8 replicates, and were repeated 2 or 3 times.

RESULTS.—Chlamydospores did not germinate in soils moistened to saturation and placed on glass rods. They germinated when the water-saturated soils were placed on excised live bean stems. Small lesions were apparent on the stems 48 hours later. These experiments repeatedly showed, however, that in both soils, not all of the chlamydospores present in the soil mass germinated, but only those located close to the bean plant or in contact with bean tissue. Soil samples taken at a distance of 2-3 mm or more from the surface of the stem contained, with very few exceptions, only ungerminated chlamydospores. The same was true of samples taken at the surface of the soil mass.

Germination of chlamydospores in close proximity with living bean tissue was followed at 6-hour intervals for 48 hours by examining stained epidermal strips. The 1st examination occurred 6 hours after the soils were placed on the bean stems, at which time no chlamydospores were germinated. A few chlamydospores had germinated after 12 hours, with the greater proportion germinating after 18 hours. Subsequent growth was rapid, and hyphal development was well advanced at 24-48 hours (Fig. 1); at 48 hours, penetration and lesion formation were observed. During this time, a few late starters germinated. Some chlamydospores had not germinated at the conclusion of the experiment. These replicated experiments showed, therefore, that all of the chlamydospores located near the surface of the host tissues (the most favorable zone for their germination) did not germinate at the same time, but rather, over a period of 6-8 hours or more after the 1st chlamydospores had germinated.

Similar replicated experiments were done with macroconidia. The spores were taken from PDA cultures, washed by centrifugation, and added to the soils at a concentration to equal 100,000 spores/g of soil. The soils were then brought up to saturation with water and placed on excised live bean stems. Germination began 6 hours later. All macroconidia had produced germ tubes of approximately equal length after 12 hours, regardless of their position in the soil mass with reference to the host tissues. It was apparent, therefore, that unlike chlamydospores, macroconidia germinate rapidly and uniformly when added to soil and their germination is not affected by the proximity of the host tissues.

The behavior of chlamydospores in soils placed on excised live bean stems also was followed in replicated experiments for 7 days. The soils were examined every 24 hours at 3 locations in the soil mass: at the soil surface, at the center of the soil mass, and at the surface of the bean stem. Four replicates were examined in this manner at each period. Germination proceeded at the stem surface as previously described. At the end of the experiment, the small discrete initial lesions on the stem had coalesced to form single large lesions and the fungus was developing vigorously in the bean tissues. The zone of chlamydospore germination remained mostly within 2-3 mm of the bean surface. No germinating chlamydospores were observed in soil samples at the surface; the numbers of germinated chlamydospores decreased as distance from the bean stem increased. After 4 days, at the surface of the bean, empty hyphae were noticed and became progressively more abundant, indicating a decline in activity of the fungus.

Since the lack of chlamydospore germination on the soil surface in the preceding experiments could have been due to a slight drying of the soil in the outer layers, these experiments were repeated using only the sandy loam soil. In 1 case, the soils were saturated

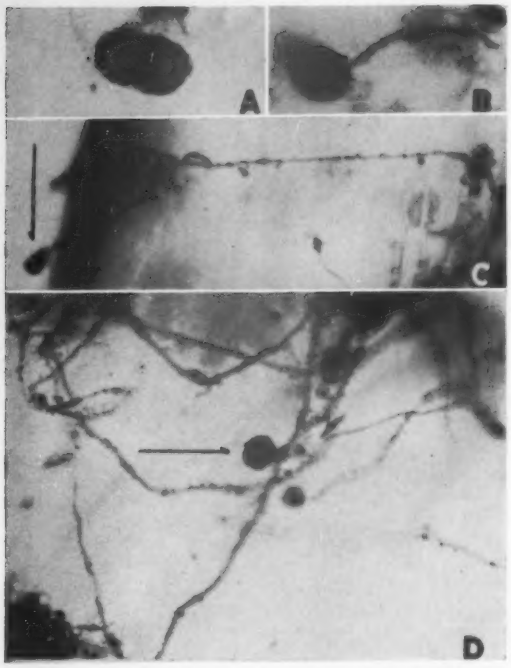

Fig. 1. Germination of chlamydospores of *F. solani* f. *phaseoli* in unsterilized soil placed on excised bean stems in petri dish moist chambers. **A)** Ungerminated 2-celled chlamydospore as it exists in unsterilized soil. **B)** Germination after 18 hours. **C, D)** Extent of mycelial development after 24 and 48 hours, respectively; arrows indicate chlamydospores.

anew with distilled water on the 4th and 5th days, while they were in place on the bean stems. In a 2nd case, Bentonite clay was added to the soil at a rate of 10% by weight and the soils were then brought up to saturation. In both of these experiments, the surfaces of the soils remained saturated for the 7-day duration of the experiment but the results were similar to those in the preceding experiments. It appears, therefore, that chlamydospores again were stimulated to germinate by their proximity to the excised live bean tissues. This activity reached its maximum in about 2 days and began to decline after 4 days, without ever reaching the layers of soils more than 2-3 mm away from the surface of the host tissues.

DISCUSSION.—Though these studies were made with soils heavily fortified with the fungus and under conditions of temperature, moisture, and host vigor favorable to pathogen growth, the results show that chlamydospores of *F. solani* f. *phaseoli* are not prone to germinate in field soil, but that they do so most readily in the vicinity of the host plant. This view is supported by the work of Schroth and Snyder (5) who suggested that chlamydospores germinated in the proximity of the below-ground parts of the plant presumably as a result of exudation from the host.

Not all chlamydospores located on the surface of the host germinated at once; rather, they did so gradually. Such behavior could possibly be due to differences in wall thickness. Macroconidia, on the other hand, germinated readily and uniformly under such conditions and did not seem influenced by the presence or absence of host tissues. This would indicate a fundamental difference in the physiology of these 2 spore forms.

Nash, *et al.* (4) have observed that macroconidia, when added to moist field soil, convert into chlamydospores either directly or indirectly through germination. Thus, experiments on the comparative behavior of chlamydospores and macroconidia were made only with recently incorporated macroconidia.

The inactivity of the chlamydospores indicates that the fungus is usually in a passive state in soil, a condition also demonstrated by Dr. D. W. Burke (personal communication). This inactivity suggests chance encounter of the host with the pathogen in the stimulation of germination. Inactivity also assures growth of the fungus only under favorable conditions and is therefore of high survival value.

The inactivity of the chlamydospores in nature recalls the observations of Dobbs and Hinson (2) on the behavior of several fungi in soil. The phenomena of fungistasis as described by these authors, however, are little understood as yet (1); it would appear, therefore, premature to ascribe the results reported on this *Fusarium* to any specific cause.

The results presented here suggest that the following 5 conditions have a bearing on the pathogenicity of *F. solani* f. *phaseoli* in soil: 1) the distance of the infection unit from the host; 2) the physiological state or wall condition of the chlamydospores which determines in part the rapidity of germination; 3) the type of infection unit (chlamydospores or conidium); 4) favorable physical environment; and 5) favorable nutrient environment (7).

Thus, the infectivity of a given soil pathogen such as *Fusarium* is related only indirectly to its population density. Therefore, attempts to measure population by the severity of the disease induced generally give lower values than plate counts, and are frequently unreliable. It also is evident that very high quantities of inoculum must be used in testing pathogenicity of fungus pathogens in nonsterile soil (3)—because all the fungus propagules do not germinate, and most of those that do are immediately adjacent to the plant which they infect, each independently, to produce multiple infections (6). It is the coalescing of multiple infection centers which results in a severe disease. Ratings based on the severity of disease resulting from a single infection would seem to be preferable to the present practice of measuring disease severity by numbers of coalesced lesions.

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EFFECTS OF STORAGE ENVIRONMENT ON "IN VITRO" HATCHING OF LARVAE FROM CYSTS OF HETERODERA SCHACHTII SCHMIDT, 1871

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SUMMARY

Freshly-reared cysts stored in sand under different environments or purified and stored at controlled humidity, were compared by *in vitro* hatch tests with standard hatch factor. Rapid drying or freezing reduced the hatch to a low number. Larvae hatched as well from cysts cleaned and stored over salt solutions as from cysts stored in sand. Dessication decreased hatching but the dessication effect may be primarily a function of rate of drying rather than degree of dryness.

Sugar beet nematode cysts may be conveniently obtained in large numbers and with increased uniformity in physiological character by rearing the nematodes upon sugar beets under greenhouse conditions (5). Successful storage of cysts would extend the advantages of nematode rearing over longer periods, thereby saving time and effort in procurement. Optimum conditions for the storage of *Heterodera schachtii* cysts have not yet been reported. Pretest environment can greatly affect the results of *in vitro* hatching (1, 2, 6, 8). Observation of such effects with cysts fresh from rearing pots would be helpful for the definition of improved storage conditions.

MATERIALS AND METHODS.—*H. schachtii* were reared on sugar beets grown in pots of sand watered with mineral nutrient (10). Some 2-3 months after infestation of the sand with *H. schachtii* larvae, when the plants manifested the characteristic symptoms of midday wilting, *etc.*, the cysts were harvested and stored in the following manner.

Group I.—Of 12 pots, 2 were set aside and allowed to dry in the greenhouse. Approximately 75% of the root systems, together with adhering sand, were removed from each of the remaining 10 plants. The plants then were repotted in sand, returned to the greenhouse, and there watered with nutrient, as before. The sand and roots removed from the plants were all combined and mixed well for uniformity of cyst and root distribution and then repotted (10 pots) and watered to settle and pack the contents. Of these 10 pots, 4 were set aside in the greenhouse and watered with nutrient to simulate plant-growing conditions; 3 were allowed to dry in the greenhouse; 2 were placed in a refrigerator at 4°C; and 1 was placed in a freezer at −18°C. After 1 month at these conditions, the cysts were separated and freed of debris by wet screening, *etc.*; and hand picked into lots of 200 for *in vitro* hatch tests with standard lyophilized hatch factor material after the method of Viglierchio (7). A separate lot of fresh cysts was drained dry on cloth towels and allowed to air-dry overnight (approximately 45% RH) for comparison in the hatch test. The cysts stored in sand at 4° and −18°C remained moist until the screening though no water had been added in the interim.

Group II.—Heavily infected plants (as previously described) from 10 pots were transferred, after approximately 75% of the roots and adhering sand were removed, to clean pots of sand, as before. The roots and sand were combined and the cysts separated immediately by wet screening. The cysts were divided into 6 equal fractions before being spread into a thin layer on separate screen dishes (60 mesh); 1 dish was placed in each controlled environment (4) of 98, 92.5, 90, 84, 80, and 75% RH at 25°C in darkness. After 1 month, the cysts from the plants were separated and compared with cysts stored at each humidity by *in vitro* hatch tests.

RESULTS AND DISCUSSION.—The response of cysts stored in sand under differing environmental regimes (Group I) measured by *in vitro* hatch tests is indicated in Fig. 1. All cysts were derived from a common source except for the lot which was allowed to dry on the plant. Evidently, moderately slow freezing and rapid drying decreased the hatch to very low values regardless of hatch factor concentration. Presumably, this occurred as a result of a corresponding decrease in viable contents (3). In other experiments, a 10-day exposure to freezing gave the same results so that even shorter exposure to low temperatures would probably decrease the hatch to very low values.

In an infestation of several generations all stages

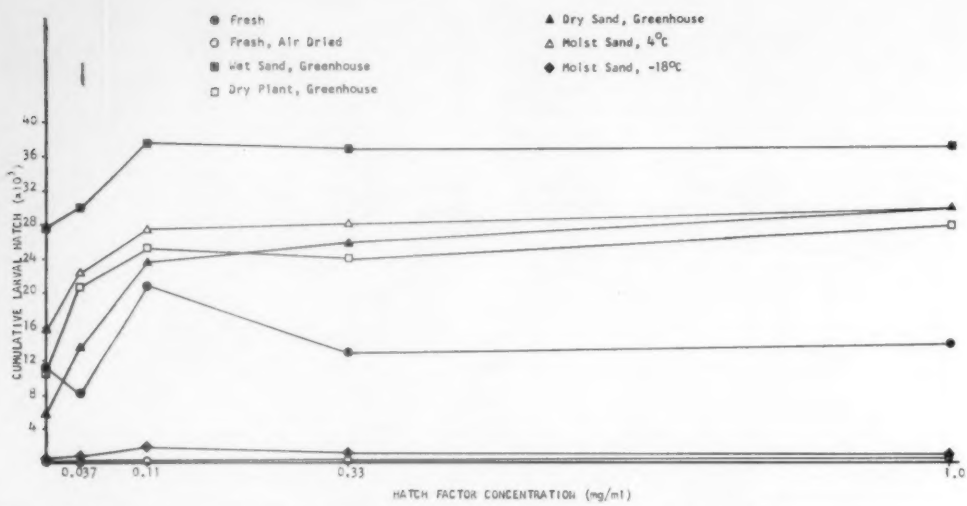

Fig. 1. The curves show the hatch response to increasing concentration of standard hatch factor for cysts stored 1 month as indicated. The points represent the average of 8 replications of 200 cysts/replicate.

of *H. schachtii* present will have been exposed continuously to varying concentrations of plant exudates. Each female or cyst will differ, therefore, in sensitivity to hatch agent depending upon the remaining number of hatchable larvae. There can result a weaker hatch response and a degree of uncertainty in the subsequent *in vitro* standard hatch assay. Unless there is some factor making for uniformity—drying, storage, hand selection, *etc.* (not present with the fresh cysts used in these experiments), an erratic response to increasing concentration of hatch agent is not surprising.

The hatching response by cysts stored in wet sand indicates that little hatching took place under a normal greenhouse watering program, though the potential was high. Wallace (9) has studied hatching and emergence of *H. schachtii* in sand and his explanations likely apply here.

The cysts stored at 4°C manifested a hatch response to increasing hatch factor concentration similar to that of cysts stored in wet sand, except for reduced cumulative larval hatches. Since the sand, initially wet, at 4°C was just moist by the end of the storage period, the general decrease in hatching may have been due to a loss of viability from drying.

There was little difference between cysts allowed to dry with or without plants as determined by *in vitro* hatch tests. The increase in sensitivity of cysts with plants at low hatch factor concentrations may have been a manifestation of the fact that these cysts were not from a uniformly mixed population as were the other lots. The results of 2 other experiments with lots of cysts reared and treated in the same manner were consistent with the interpretation of these data.

The hatching response with increasing hatch factor concentration of cysts stored at predetermined RH (Group II) is shown in Fig. 2. Cysts stored at 92, 92.5, and 98% RH provided good *in vitro* hatch responses to increasing hatch factor concentration. Cysts stored at 84% RH showed a much lower hatch. Cysts stored at 75 and 80% RH showed essentially the same hatch but less than that at 84%.

The relative increases in hatching due to increasing hatch factor concentration of cysts stored in sand are comparable to those of clean cysts stored in humidity chambers. The high hatches from cysts in sand are a selection effect, for in other experiments the cyst hatches were of the same order of the cysts in humidity chambers. It is possible, therefore, to rear sugar beet nematodes and harvest the cysts immediately for storage at controlled humidity without undue loss of reactivity to hatch factor.

Drying of cysts (8) reduces the viability of the contents; the data presented here are consistent with this finding. It appears, however, if all the data are considered, that the dessication effect is primarily a function of rate of drying rather than degree of dryness. The close grouping of hatch responses of cysts stored in moist sand at 4°C, dry sand, or on a dry plant (Fig. 1), as well as the similar responses of cysts at 98, 92.5, and 90% RH, and cysts at 80 and 75% RH, would be better explained as a rate of drying effect.

Large numbers of cysts were placed in the humidity chambers and apparently the rate of drying was slower than that reported by Wallace (8). This explanation also would be more consistent with the events taking place in nature; *e.g.*, the dessication of cysts in soil and stem and bulb nematode in plants are slow processes. Presumably, the gradual change of environment provides the larvae within the egg casing greater opportunity to mobilize solutes, reduce metabolism, and otherwise adapt to adverse conditions enabling it to survive during extended periods of stress.

It also appears that slow drying improves the re-

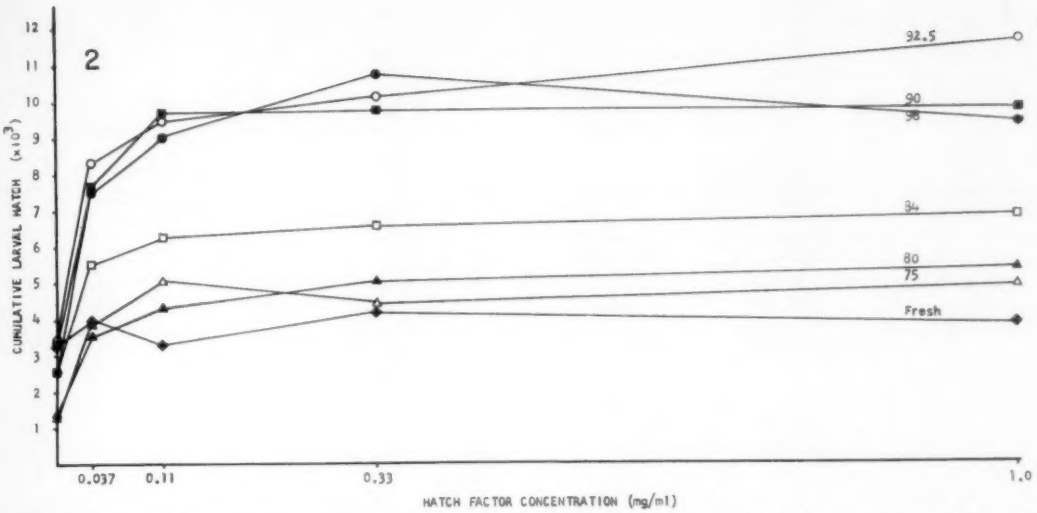

Fig. 2. The curves show the hatch response to increasing concentration of standard hatch factor for cysts stored 1 month at the humidity indicated. The points represent the average of 8 replications of 170 cysts/replicate.

producibility of the *in vitro* response to hatch-factor stimulation. The mechanism by which such action is manifested needs further study.

If the results with slow freezing and rapid drying could be extended to field application, either slow freezing or rapid drying of wet cysts could be incorporated into a rotation program in suitable areas. The benefits of such programs, however, would need evaluation by appropriate tests.

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COLONIZATION OF ORGANIC MATTER IN THE SOIL BY FUNGI

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SUMMARY

Layers of sterilized wheat, oat, or barley straw were placed in pots of soil from 24 fields. *Penicillium* spp., *Rhizopus nigricans*, and *Aspergillus niger* were the main fungi recovered by dilution plating. Their numbers differed markedly in relation to kind of straw and the soil sample. Cereal seedlings grown in sterilized straws placed in unsterilized soil were severely damaged by the fungi that invaded the straw. Oat hulls and corn meal were unsatisfactory as substrata in making inoculum of

Helminthosporium sativum, because of the heavy seedling damage in the controls. No damage occurred when the seed was planted in sterilized peat. The dominant fungi in this medium were *Penicillium* spp., *R. nigricans*, and *Trichoderma viride*, whereas *Penicillium* spp. and *R. nigricans* were dominant in straw. Evidence was secured of differences among the various fungi in mobility and in competition.

INTRODUCTION.—In greenhouse trials of seed treatments for the control of cereal root disease caused by *Helminthosporium sativum* P. K. & B., infection was less in sterilized than in unsterilized soil. Sphagnum peat was included in the potting mixture to improve texture and retention of moisture; this addendum might have been responsible for the decrease of infection. Similar phenomena had been noted earlier, relative to various cereal straws in soil; microflora involved in decomposition differed according to the kind of straw added (10, 11). Zogg (12) recently reported that the pathogenicity of *Ophiobolus graminis* was lost when the rhizosphere microflora of clover was added to the culture, whereas no loss of pathogenicity occurred when the rhizosphere microflora of wheat was added.

It seems that in root disease the pathogens may be considerably influenced by the other flora present. Such effects, however, are not confined to interfungal relationships. The plant itself may be adversely affected by soil saprophytes under certain conditions. Hildebrand and West (5), in a study of strawberry root rot caused by *Thielaviopsis basicola*, found a lack of correlation between the presence of the fungus and discolored or necrotic tissue on the host. Different kinds of plant debris had been applied to the root rot soil. It appeared that some species of the soil microflora favored by certain debris were capable of either directly attacking strawberry roots or of injuring them by toxins. Edwards (4), in a study of corn, found that seedlings from seed inoculated with *Gibberella fujikuroi* were healthier than the controls. The latter were heavily overrun by *Trichoderma viride* which could not be detected in the former. Ludwig, *et al.* (6) and others have shown that plant pathogens may form phytotoxic compounds but it is apparent that saprophytes also are capable of producing phytotoxins.

Experiments were planned to assess the effects of the interaction between saprophytes and pathogens in the presence of various organic materials in the soil. The following have been considered: the effect of various types of organic matter on the kinds, numbers, and sequential development of fungi; the effect of soil saprophytes on plant pathogens; and the effect of fungitoxins on plants.

MATERIALS AND METHODS.—Mature straws of wheat, oats, and barley were ground in a hammer mill to 1-5-mm particles. The peat used was dark brown sphagnum type, sieved to about the same size as the straw. Water was added in the ratio of 2.5:1.0 by weight and when sterilization was required it was effected by steam at 15 lb for 1.5 hours in 200-ml flasks.

In experiment 1, various types of black loam from 24 different fields were employed. In all other experiments, black Edmonton loam, pH 6.2, was mixed 3:1 with sand. Metal boxes, 18 in. high x 12 in. wide x 2 in. thick and faced on 1 of the major sides with glass, were used in experiments 3, 4, 6, and 7. These were filled to a depth of about 14 in. with soil, a 1-in. layer of straw or peat treated in various manners was added, and this was covered with 3 in. of soil. In several instances, Gem barley was planted within the layers of straw or peat. The boxes were inclined at about 25° so that the barley roots grew downward against the glass surface. Certain species of fungi that colonized the straw also were identified by direct observation through the glass.

Where plating procedures were employed, the dilution used was 1 of straw as sampled to 200,000 water in 9-cm petri plates. The medium was as follows: potatoes, 300 g; dextrose, 15 g; Difco yeast extract, 2.25 g; agar, 15 g; water to 1 liter. Thirty ml of 5% lactic acid/liter were added after sterilization.

Fungi were cultured on straw, peat, or other organic substrata in 200-ml flasks under bell jars at 70-75°F for 10-14 days.

RESULTS.—1) *Fungi in various field soils.*—Samples of soil were secured from 24 fields in a local township and were placed in pots in the greenhouse. A 1-in. layer of moist sterilized ground straw of wheat, oats, or barley was added 2 in. below the surface of the soil. After 1 week, samples of the straw were removed and dilutions were added to petri plates. The numbers and predominant kinds of fungi were recorded daily for 5 days (Table 1).

Mucorae were recovered most abundantly from the wheat straw, but these fungi invaded the 3 straws in all soil samples more or less freely. *Penicillium* spp. were found in the greatest numbers in wheat and barley straw, but occurred in only half of the samples of oat straw. The data indicate the extent of the variability of soils in harboring these species and also demonstrate the relative frequency of occurence of fungi on the 3 kinds of crop debris.

2) *Growth of barley seedlings in layers of straw placed in soil.*— Wheat, oats, and barley seed were planted in layers of sterilized or unsterilized wheat, oat, or barley straw buried 2 in. deep in pots of sterilized or unsterilized soil. Treatments were replicated 4 times. After 6 days, the seedlings were removed and the growth of roots and shoots was recorded in cm (Table 2).

The 3 cereals grew well on sterilized wheat straw and in all treatments containing unsterilized wheat, oat, and barley straw, but growth was inhibited on sterilized oat and barley straw. Oats were very strongly inhibited in the 2 latter treatments and most of the seedlings were killed. Damage was greatest when

TABLE 1. Mucorae, *Penicillium* spp., and *Aspergillus niger*/g of straw that had been sterilized and placed for 1 week in pots of soil from 24 different fields [a]

Soil sample	Wheat straw			Oat straw			Barley straw		
	Mucorae	*Penicillium* spp.	*A. niger*	Mucorae	*Penicillium* spp.	*A. niger*	Mucorae	*Penicillium* spp.	*A. niger*
1	72	15		3			5		
2	55	48		25			0	10	
3	77	5	33	20	2	10	10	3	
4	202	23	63	28	15		5	25	
5	43	43	3	13	5		0	10	
6	37	42		17	5		1	37	
7	182	258	15	17			2	55	
8	105	38	2	7			28	233	
9	43	28		22	2		15		
10	50	55	13	52		13	16	37	
11	172	2	8	60	5	10	12	8	
12	70	3		35			10	13	
13	100	2	3	12			11	7	
14	43	35	3	2			18	40	
15	130	27		25	2	7	70	10	
16	225		48	18	10	3	18	8	
17	175	10	5	58		275	27	3	5
18	78	37		30	5		67	6	
19	200	38		168			72	18	8
20	42	25		22			3	18	
21	102	8		23	3		10	67	
22	70	18		45	192	17	23	6	
23	125	87		48	12	2	108	10	
24	267	12	5	108	3	27	128		

[a] Figures in table should be multiplied by 10,000.

the layer of sterile straw in which the seeds were planted was placed in unsterile soil.

Phytotoxic effects could be caused by fungi and other organisms that enter the straw from the soil or by those that are harbored in the seed. The former become established very rapidly after adding sterilized straw to unsterilized soil; damage to seedlings was greatest in these treatments. There also was marked inhibition of seedling growth in sterile straw added to sterile soil and in the controls of sterile straw in flasks. The organisms that recolonize the sterile soil in the uncovered pots would be effective in the 1st case; in the latter case, only the organisms contained in the seed could be operative. Other experiments here have shown that sterilized straw that remains uncolonized by microorganisms is harmless to cereal seedlings. It was possible to demonstrate this by placing sterile seedlings rather than seeds within the medium. Our method of surface sterilizing seed, in this and certain of the following experiments, involved presoaking in water for 4 hours, followed by soaking for 1 minute in 0.1% aqueous mercuric chloride, and repeated rinsing with sterile water. Following this treatment, about half the seeds produced colonies of various fungi when incubated on nutrient agar for 1 week.

Several theories might explain why all cereals suffered less inhibition in sterilized wheat straw placed in unsterilized soil: different species occurred in quantitative difference on the various straws; sterilized oat and barley straws offered more favorable media for the growth of microflora that are phytotoxic; different toxins were formed by the action of organisms on the different straws. The writer does not believe that the toxins operative in seedling inhibition are absorbed from the straw directly, but rather that they affect the plants from mycelium and spores in contact with the roots. Some evidence has been presented in previous work in favor of the 1st 2 theories above, and phenomena related to the addition of organic matter to the soil are reviewed (10, 11).

3) *Histology of barley roots grown in inocula of various fungi.*—Ground mature straw of wheat, oats, and barley was moistened and sterilized in flasks. Spores of *H. sativum*, *Cephalosporium* sp., or *Penicillium* sp. were added and the flasks were incubated for 10 days at room temperature. A layer of the infested straw was placed on the surface of unsterilized soil in metal boxes with 1 glass side; barley was seeded in the straw layer. Root growth was recorded after 6 days (Fig. 1). The fungus colonies were recorded daily for a week (Table 3).

Fig. 1 illustrates several interesting effects. Growth of the barley was inhibited most strongly in the uninoculated straw controls and least in the straw inoculum of *H. sativum*. Inhibition by *Penicillium* sp. and *Cephalosporium* sp. was greater in oat and wheat straw than in barley straw. In all cases where severe inhibition occurred, only stumps of roots developed, dark brown in color, and frequently clubbed. Samples of the injured portions of the roots in all treatments were fixed, embedded, sectioned, and stained. In the *H. sativum* series, the cortical cells were often so severely disintegrated that no cell organization could be distinguished nor could mycelium of the pathogen be seen. In less severely affected areas, mycelium

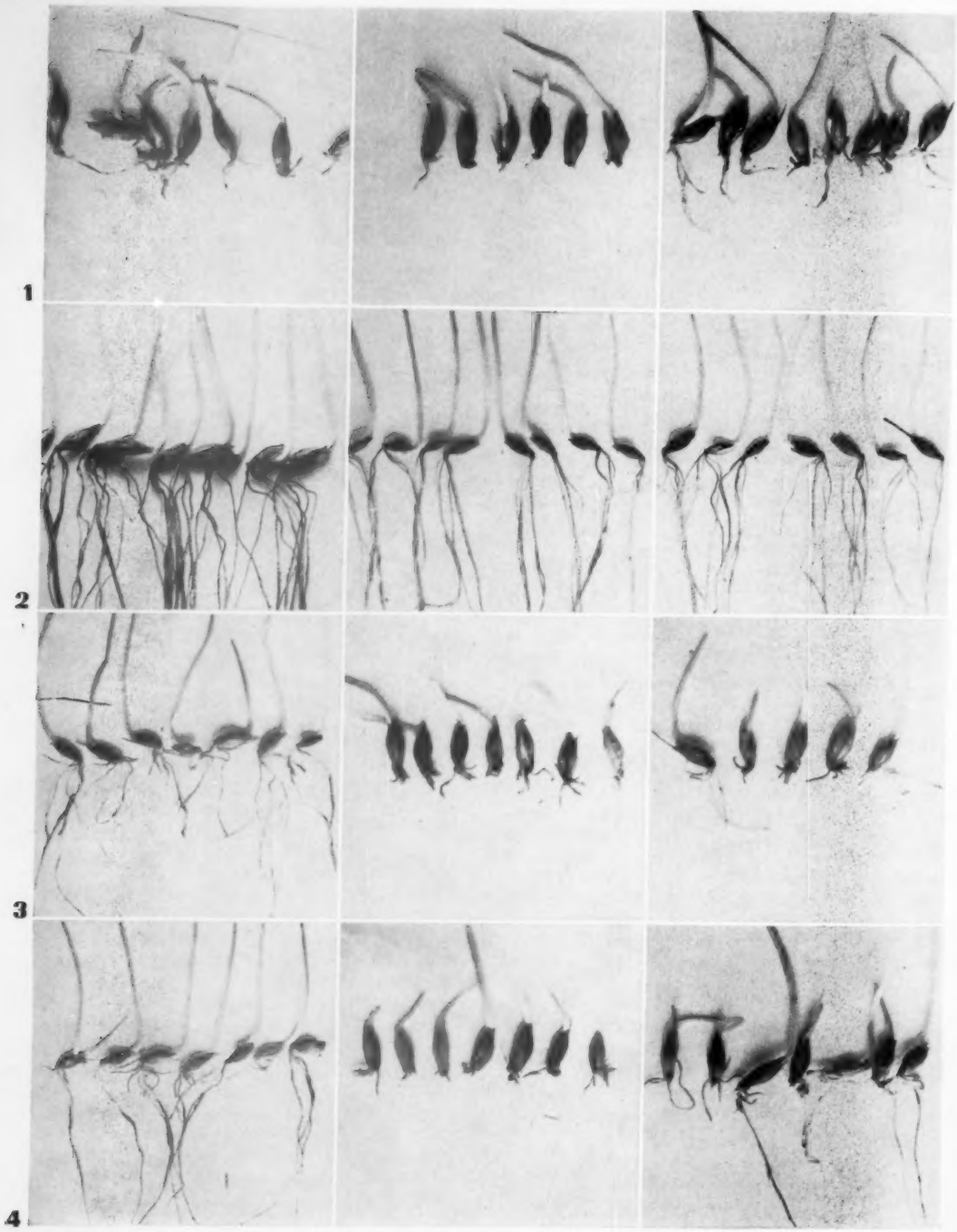

Fig. 1. Growth of barley after 5 days in layers of barley, oat, and wheat straw under unsterile soil (l. to r.): **1)** sterile straw controls; **2)** straw inoculum of *Helminthosporium sativum;* **3)** straw inoculum of *Penicillium* sp.; **4)** straw inoculum of *Cephalosporium* sp.

TABLE 2. Growth in cm of shoots and roots of cereals 6 days after planting in layers of straw placed in pots of soil

Seed	Soil	Wheat straw		Oat straw		Barley straw		Soil control
		Ster.	Unster.	Ster.	Unster.	Ster.	Unster.	
Wheat	Ster.	6.9	7.3	2.7	9.5	3.2	10.2	10.8
	Unster.	5.7	9.7	1.4	9.7	1.2	7.4	12.6
	None	7.7	20.0	2.8	14.2	4.9	15.0	
Oats	Ster.	6.8	7.8	0.6	6.3	1.5	5.0	7.4
	Unster.	5.1	8.7	0.3	4.7	0.5	6.3	11.9
	None	3.0	14.9	0.6	9.1	1.0	7.2	
Barley	Ster.	8.4	12.4	4.9	11.7	6.0	11.5	14.2
	Unster.	5.6	11.7	1.3	12.5	2.5	11.3	17.8
	None	10.7	19.0	5.4	19.7	7.9	14.2	

was frequently observed. It was found in only 1 case within the endodermis; however, no evidence of cell disintegration was noted. It appears that toxins were indeed operative in effecting necrosis, and that these were absorbed by the roots from internal and external mycelium.

In the *Cephalosporium* series, fine internal mycelium was found in a great many instances. No internal mycelium was found in roots of the control or *Penicillium* series, but disintegration of the cortex was extensive. It seems that toxins were mainly concerned in effecting necrosis in the control series and also in the *Penicillium* and probably in the *Cephalosporium* series. Fungi such as *Rhizopus nigricans* Ehrenb. ex Fr. invaded the straw of the control series from the soil within 1 day and also invaded the straw of the *Penicillium* and *Cephalosporium* series to some extent, but not the straw inoculum of *H. sativum*, which apparently repels invaders. Additionally, barley seedlings were placed in straw or peat infested with inoculum of *Aspergillus niger* van Tiegh., *R. nigricans*, *Trichoderma viride* Pers. ex Fr., and *Penicillium* sp. for 5 days; sections of the roots prepared as above were examined. Internal mycelium of the fungi was not found but sporulation on the root surface was very heavy. The cortical cells were severely disintegrated. In the *R. nigricans* series, rhizoids were once observed within the 1st few layers of cells.

4) *Relative toxicity of various fungi to barley roots.*—In a further experiment, inocula of *A. niger*, *T. viride*, *Cephalothecium roseum* Corda, and *R. nigricans* were prepared in sterilized straw of wheat, oats, and barley. Barley was planted within layers of these inocula and covered with unsterilized soil in glass-faced boxes. Growth of the roots after 5 days is shown in Fig. 2. *A. niger* and *T. viride* were very destructive. These may be of more importance in the present experiments than is indicated by the population as measured by dilution plates. They do not sporulate as rapidly as do many of the Mucorae; colony counts may give a misleading indication of their incidence.

Various fungi and bacteria are harbored beneath the seed coat and escape destruction by chemical sterilants. These, however, should be of little significance in cases as above where the straw in which the seeds were planted is already heavily populated by the inoculant and where data were secured after a 5-day growth. Further studies will be undertaken on the development of internally borne fungi in the microbiological community of the soil.

5) *Effect of kind of organic matter added to the soil on the growth of barley.*—Ludwig, *et al.* (6) presented data showing the effect of type of inoculum on the pathogenicity of *H. sativum*. No controls were included, however, and, in view of the above results, it

TABLE 3. Fungi/g of straw-grown inoculum of *Penicillium* sp., *Helminthosporium sativum*, and *Cephalosporium* sp. 6 days after placing it in soil[a]

Inoculum	Fungi recovered				
	Penicillium spp.	*H. sativum*	*Cephalosporium*	*A. niger*	Mucorae
Wheat straw					
Penicillium	3000.0				3.2
H. sativum	392.0	140.0			
Cephalosporium	280.0		256.0		3.2
Control	13.2			460.0	560.0
Oat straw					
Penicillium	5666.0				0.66
H. sativum	580.0	532.0			
Cephalosporium			316.0	560.0	
Control				30.0	760.0
Barley straw					
Penicillium	4080.0				
H. sativum	240.0	40.0			3.2
Cephalosporium			383.2		6.6
Control	226.0			142.0	470.0

[a] Figures in table should be multiplied by 10,000.

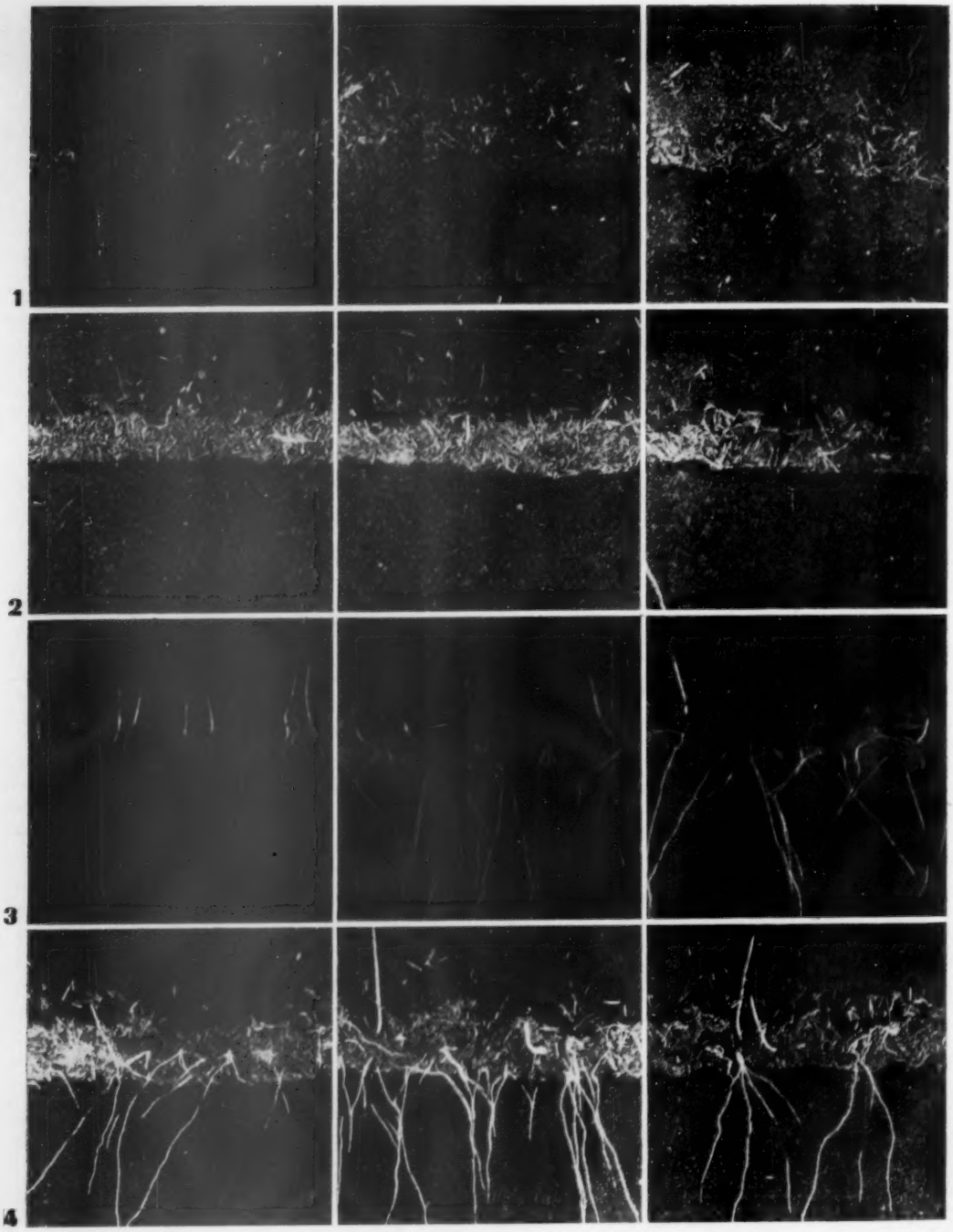

Fig. 2. Growth of barley after 5 days in layers of barley, wheat, and oat straw under unsterile soil: **1, 2, 3, 4)** straw inoculum, respectively, of *Aspergillus niger, Trichoderma viride, Cephalothecium roseum,* and *Rhizopus nigricans.*

TABLE 4. Effect of type of inoculum of *Helminthosporium sativum* on disease on barley seedlings

Type of inoculum	% soil infestation	% disease		Avg. length/cm	
		Roots	Shoots	Roots	Shoots
Soil	5	13	0	9.3	11.6
	5 control	0	0.5	12.3	11.9
	15	5	0	10.3	11.5
	15 control	0	0	11.5	12.9
Soil + 10% corn meal	5	37	20	8.7	8.6
	5 control	10	0	12.3	11.6
	15	44	25	3.6	5.3
	15 control	10	0	10.4	12.1
Corn meal	5	53	35	1.3	2.9
	5 control	59	2	1.8	7.7
	15	100	77	0.1	0.8
	15 control	62	3	2.4	6.3
Wheat	5	34	18	8.6	10.3
	5 control	24	7	6	8.2
	15	57	42	7.3	10.4
	15 control	28	5	5.6	10.2
Oat hulls	5	66	20	2.2	3.8
	5 control	63	11	1.4	6.2
	15	92	47	1.8	2.7
	15 control	69	12	1.3	6.2
None		0	0	12.1	11.1

was thought desirable to repeat their experiment. *H. sativum* was added to flasks of soil, soil plus 10% corn meal, corn meal, wheat, and oat hulls. After a 10-day incubation, the inocula were mixed with unsterilized soil in proportions of 5 or 15% by weight. Barley was seeded in 3 x 3-in. plant bands containing these mixtures. The controls were sterilized, identical substrata, lacking the pathogen. The data on growth and infection after 9 days appear in Table 4.

The control treatments employing corn meal or oat hulls alone caused very severe root damage and inhibition of growth which must be attributed to the soil microorganisms that rapidly colonize the particles of organic matter added. Inhibition of growth and root damage also was greatest in those media inoculated with *H. sativum;* these effects were consistently greatest where the soil received 15% inoculum. Thus, an important part of the damage may be attributed to soil flora other than *H. sativum*. The use of excessive organic matter in the preparation of inocula and diluting it in unsterilized soil are thus contraindicated procedures. Sallans (8) also reported difficulty in obtaining reliable infection data of wheat seedlings by adding oat-hull inoculum of *H. sativum* to unsterilized soil.

In experiment 3, the effect of *H. sativum* could be isolated from that of other organisms by seeding within a layer of inoculum unmixed with soil. Ludwig, *et al.* (6) also suggested that the most satisfactory infection by *H. sativum* can be achieved by placing inoculum both above and below the seed. Apparently, the saprophytes that attempt to establish themselves in the sterile organic matter in the inoculum cannot penetrate the layer before the pathogen has been effective.

6) *A comparison of straw and peat as substrata for the growth of fungi.*—Efforts were made to determine the sequence of fungi that colonize straw and peat over a period of 2 weeks and to relate the findings to the effects of toxins on roots as noted above. Sterilized and unsterilized oat straw, wheat straw, and peat were layered in soil in glass-faced boxes. No seeds were planted; samples of the straw were secured for plating as above at 4, 7, 10, and 14 days after setting out (Table 5).

Mucorae, mainly *Rhizopus nigricans,* were readily observed through the glass invading the sterilized oat and wheat straw after 1 day. These were the dominant species at the 1st plating and increased in the subsequent platings. Mucorae were relatively much less numerous in peat and in unsterilized straw. *Penicillium* spp. were fairly well represented in all treatments and especially in sterilized peat. *Trichoderma viride* occurred only in peat, but it is not known whether its presence suppressed the growth of *Penicillium* spp. and Mucorae or that peat is an inhospitable medium for them. *Cephalothecium roseum, Fusarium,* and *Chaetomium* spp. were not found in sterilized straw and it is possible that they are poor competitors of *Penicillium* spp. and Mucorae, at least during the period considered. Various additional species of fungi occurred occasionally, but the above-named genera or species were the same as those found most prominent in corn seed from several American states by Edwards (3).

Additionally, soil boxes prepared as above were planted to barley and the roots were photographed after a 5-day growth (Fig. 3). The extremely damaging effects in sterilized wheat and oat straw are in sharp contrast to the lack of inhibition in the sterilized peat. Table 5 shows a different sequential development of fungi in the 2 types of media; this may be of significance in the effects observed on the plants. Water extracts of peat and straw inocula of the various fungi considered showed no differential effects on the growth of barley roots in test-tube culture.

7) *Mobility of various fungi in the soil.*—Cheese-

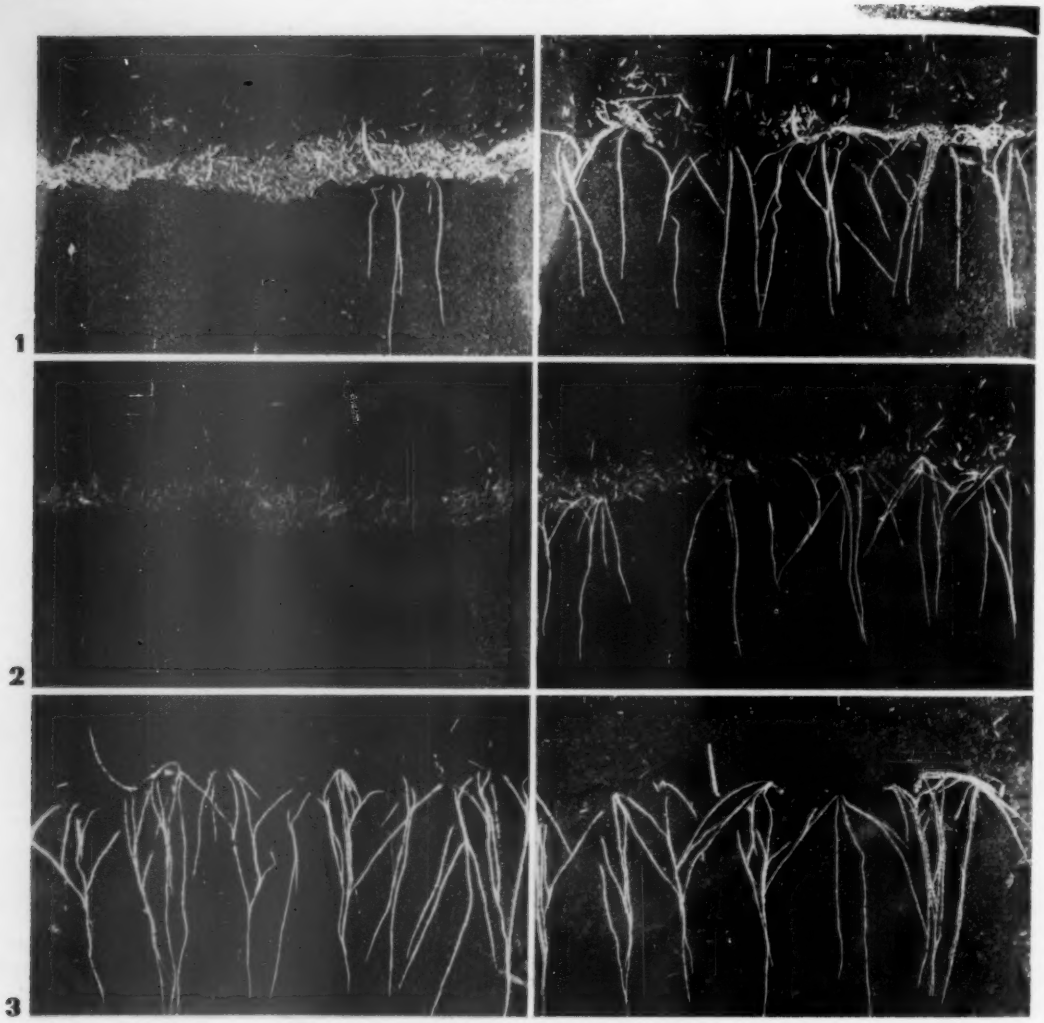

Fig. 3. Growth of barley, sterilized and unsterilized, after 6 days in layers of straw or peat under unsterile soil. Rows 1, 2, 3) wheat straw, oat straw, and peat.

cloth bags, 12 x 2 in., were filled with inocula of various fungi cultured on wheat, oat, or barley straw and covered with unsterilized soil in the soil boxes. A bag of *H. sativum* inoculum was placed in each box and a bag of inoculum of *T. viride*, *R. nigricans*, *A. niger*, or *Penicillium* sp. was placed above it. After 1 week, the bags were removed, opened, and dilutions made for plating. Colonies of fungi were counted and identified. All straw samples were examined microscopically to determine the presence of spores of the fungi indicated.

The various fungi differed in their ability to invade the inocula of each other. *H. sativum* readily invaded the inocula of *Penicillium* spp. or *R. nigricans* but it was not found in the inocula of *A. niger* or *T. viride*. *A. niger* was the most aggressive competitor and was found in the inoculum of *H. sativum* placed adjacent to it, and also in the inocula of *Penicillium* sp., *T. viride*, and *R. nigricans* which it must have entered from the soil. The latter 3 fungi did not invade inocula of each other nor that of *A. niger* from the soil, but it is possible that the cheesecloth bags presented barriers to their mobility. They also did not invade inoculum of *H. sativum*, either from the soil or from adjacent bags; however, traces of *Penicillium* were found associated with *H. sativum* in 3 cases, 2 of which were invasions from the soil.

Thus, the soil saprophytes varied in mobility, in sequential development, and in their ability to compete with *H. sativum* over the period concerned. The pathogen was a successful competitor and was difficult to displace over long periods. Butler (1) reported that

TABLE 5. Fungi/g of sterilized and unsterilized wheat straw, oat straw, or peat buried in soil for various periods [a]

Treatment	Plated after (days)	Mucorae	*Penicillium* spp.	*Fusarium* spp.	*C. roseum*	*Chaetomium* spp.	*T. viride*
Wheat straw, sterilized	4	377	7	0	0	0	0
	7	833	3	0	0	0	0
	10	186	17	0	0	0	0
	14	1067	43	0	0	0	0
Wheat straw, unsterilized	4	13	7	10	17	13	0
	7	67	0	10	257	13	0
	10	0	7	3	1533	20	0
	14	3	20	20	4667	10	0
Oat straw, sterilized	4	340	7	0	3	0	0
	7	600	0	0	0	0	0
	10	433	10	0	0	0	0
	14	587	7	0	0	0	0
Oat straw, unsterilized	4	30	13	7	10	0	0
	7	27	20	7	1557	10	0
	10	7	10	3	1833	0	0
	14	0	7	0	1166	7	0
Peat, sterilized	4	37	93	3	0	0	0
	7	47	93	0	0	0	17
	10	80	100	0	0	0	20
	14	97	20	0	0	0	20
Peat, unsterilized	4	13	27	0	0	0	0
	7	10	10	0	0	0	0
	10	0	20	0	0	0	3
	14	57	40	0	0	0	20

[a] Figures in table should be multiplied by 10,000.

H. sativum was only moderately successful in competing as a saprophyte. In his experiments, straw was placed in mixtures of inoculum of the pathogen with unsterilized soil, whereas in the present work *H. sativum* was first established in straw which was then placed in the soil. The latter conditions would prevail in nature in the case of stubble that has been infected by the pathogen during the crop season.

DISCUSSION.—Saprophytic fungi of the soil can cause damage to cereal seedlings that simulates damage caused by recognized pathogens. This fact is of more than academic interest, since any inoculum of high organic matter content as well as the control substratum will favor intensive growth of these fungi. The damage caused can thus be superimposed upon that incited by the pathogens and lead to erroneous conclusions.

No toxic effect appeared on cereal roots from the sterile organic matter itself. Phytotoxins were produced by the fungi that colonized this substratum and supposedly sterile seeds were often the source of the fungi. Damage was not experienced in unsterilized organic addenda; it seems that the microorganisms present impeded aggression by any 1 of them. Sterile substrata, on the other hand, were quickly overrun by the 1st fungus to invade. In this connection, Piening (7) has pointed out that some danger is attendant on the practice of partially sterilizing greenhouse soils, particularly if it is likely that pathogens may be reintroduced soon after the sterilizing operation.

A sequential development of species was operative, and these differed in kind and number according to the substratum used. Such differences existed between straws and peat and may partially explain the marked differences in growth of seedlings in these media. A similar sequence of ascendancy was reported by Chesters and Parkinson (3) on living oat roots. Mucorales developed rapidly at first but these were soon supplanted by a mixed population of fungi. *Penicillium* and *Trichoderma* spp. were present in significant numbers, however, at all stages from seedling to senescence.

Damage to seedlings, such as necrosis of roots, and inhibition of growth of roots and shoots caused by fungus saprophytes, may be attributed to toxins absorbed from external mycelium. This was not strictly true, however, since mycelia of some of these fungi were found occasionally within the root tissue. Where the saprophyte exists in unrestricted abundance, it seems to act as a pathogen. Conversely, in unsterilized media, no deleterious effects on plant roots were noted as this environment supports a multiplicity of organisms that presumably keep each other in quantitative balance. *H. sativum* is notably pathogenic under intensive wheat or barley cropping, but is significantly reduced by fallow or oat crops in the rotation. Under wheat or barley, it apparently becomes the ascendant fungus; whereas, under oats, it is subject to restraint by other microorganisms of the soil community.

Simmonds, *et al.* (9) readily secured infection of wheat and barley by adding a suspension of spores of *H. sativum* to the soil; whereas Ludwig, *et al.* (6) were unable to obtain infection by this procedure. This is only an apparent contradiction if the above theories are accepted. In 1 case, the pathogen was not impeded in establishing itself; but, in the 2nd case, a microbiological population inimical to *H. sativum* was present.

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PHYTOPHTHORA MEGASPERMA, THE PRINCIPAL CAUSE OF SEED-PIECE ROT OF SUGARCANE IN LOUISIANA

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SUMMARY

A study was made of *Phytophthora* seed-piece rot of sugarcane in Louisiana. The principal cause of this disease had been known for several years as a so-called sterile isolate of an unidentified *Phytophthora* sp. In this study the organism produced fruiting structures when grown on sterilized oat grains. After comparing cultures of seemingly related *Phytophthora* spp. (*P. megasperma, P. drechsleri, P. cryptogea,* and *P. cinnamomi*), in respect to morphology, growth-temperature relations, and pathogenicity, this isolate was identified as a small-spored form of *Phytophthora megasperma.* Sporangia (27 x 42 μ) are borne on long sporangiophores with abundant proliferation; are ovoid to bluntly ellipsoid in shape; and have inconspicuous papillae. Germination is occasionally direct, but most frequently by production of zoospores. Chlamydospores (36 μ) are intercalary, germinating with 1-7 germtubes. Oogonia (35 μ) are spherical, with the antheridia usually paragynously attached to them. Oospores (31 μ) are plerotic with a thick endospore. The isolate grows in the range 5-23°C, and optimally at 20-22°C. The isolate made fair growth even after 30 days' incubation at the minimum temperature, indicating its ability to survive during prolonged cold weather. In pathogenicity tests on sugarcane seed pieces, *P. megasperma* was about twice as virulent as *P. erythroseptica.* This disease can now be added to the short list of plant diseases caused by 2 *Phytophthora* spp.

Introduction.—*Phytophthora* seed-piece rot, a minor disease of sugarcane (*Saccharum officinarum* L.), has been recorded only from Louisiana. The principal disease of seed pieces in Louisiana is red rot, caused by *Physalospora tucumanensis* (Went) Speg. (1). Typical symptoms of these 2 seed-piece rots have been clearly described (39).

In 1947-48, Chilton and Steib (10) observed stand failures of variety C.P. 36-13 in Louisiana. This variety was highly resistant to red rot, resistant to

mosaic, and moderately resistant to root rot, caused by *Pythium arrhenomanes* Drechs. (7). Therefore, some other disease was suspected as being responsible for the stand failures.

A few years later, Steib and Chilton (34) reported that of 2,017 nodes and internodes plated, 51.5% gave *Phytophthora* isolates. These isolates were divided into 3 distinct cultural types—strains 1, 2, and 3.

Strain 1, characterized by production of oogonia and amphigynous antheridia on oatmeal agar and abundant sporangial production, was identified as *Phytophthora erythroseptica* Pethyb.; strain 2, characterized by absence of sexual structures and production of few sporangia, was called the sterile mycelium type and was the most pathogenic; strain 3, characterized by production of only paragynous antheridia and sporangia as in strain 1, was called the *Pythium*-like *Phytophthora* and was least pathogenic.

Sanchez-Navarrette (30) first attempted to induce formation of fruiting structures in the sterile *Phytophthora* isolate, by crossing the isolate with several *Phytophthora* spp., but all his trials failed. Singh (32), a few years later, made further attempts by using different media, fleshy fruit, soil leachate, sugarcane stalks and roots, light manipulation, and paired cultures. Of all the materials used, only the inoculation of sugarcane roots resulted in a limited production of sporangia and oogonia. Abundant production of chlamydospores was observed by both investigators.

The present study was undertaken: 1) to make new attempts to induce fruiting in the sterile *Phytophthora* strain; 2) to make comparative studies of this isolate with closely related species of *Phytophthora* in regard to morphology, growth-temperature relations, and pathogenicity; and 3) to identify this principal causal organism of sugarcane seed-piece rot.

Materials and Methods.—Since previous investigators of seed-piece rot (30, 32, 34) did not report examination of the fungus on oats, the inoculum then used for soil-infestation and seed-piece inoculation studies, we considered sterilized oats as a possible fruiting medium.

The fungus was grown on sterilized oats in Erlenmeyer flasks; this inoculum was intended for field tests by planting with seed pieces in the furrow. Flasks were stored in a constant-temperature room at 21°C, and the oats were examined at intervals for fungus fruiting bodies. Oat grains with the fungus also were placed in petri dishes with tap water to test sporangial production, simulating a method recently used by Lowy (24) in the study of aquatic Phycomycetes. This technique was modified by placing the oat grains on wooden rods held with cotton plugs in Erlenmeyer flasks with water. This allowed the fungus to grow out in all directions.

The sterile sugarcane isolate was compared with *P. cinnamomi* Rands from avocado, *P. erythroseptica* Pethyb. from potato, *P. drechsleri* Tucker and *P. megasperma* Drechs. from unknown hosts (obtained from the American Type Culture Collection), and *P. cryptogea* Pethyb. & Laff. from alfalfa, kindly supplied by D. C. Erwin, Citrus Experiment Station, Riverside, Calif.

All these *Phytophthora* spp. and the sugarcane isolate were compared at 5°, 10°, 15°, 20°, 25°, 27°, 30°, and 35°C. The isolates were grown in similar-sized petri dishes with 20-ml potato-dextrose agar (PDA), by placing a 12-mm plug of each culture in the center of the dish. The plates were incubated at various temperatures, and colonies measured 6, 10, and 30 days later.

The *Phytophthora* spp. which most resembled the sugarcane isolate morphologically were tested on 5 additional culture media at 10°, 20°, and 32°C, to compare any differences in growth. The inocula were 10-mm plugs from PDA cultures. Oatmeal, cornmeal, lima-bean, Czapek's, and Fries' agars, made with distilled water, were used (13, 23, 29). Colony diameters were measured after 6 days of incubation. Both temperature experiments were run with 4 replications each, and the experiments were repeated once.

P. megasperma and the sugarcane isolate also were compared as to pathogenicity on apples, eggplants, and oranges, fruits used by Tucker (37) and Wager (41) in their comparative studies of *Phytophthora* spp. These fruits were surface-sterilized by dipping in 1:1,000 mercuric chloride solution and then placed in moist chambers. Inoculations were made by placing a 12-mm agar disc with mycelium into a small, shallow wound in the fruit surface. Check fruits also were wounded. The moist chambers then were incubated at 21°C, and the fruits were examined after 7 days.

These 2 *Phytophthora* isolates also were tested for pathogenicity on sugarcane seed pieces. Since *P. erythroseptica* had been described from seed pieces before, this species also was used in the comparative test. All 3 *Phytophthora* cultures were inoculated into a total of 10 two-eyed pieces of 2 sugarcane varieties, by placing about 5 oat grains with the fungus in a hole cut into the internode of each seed piece. The seed pieces then were planted in sterilized soil, in large crocks in a constant-temperature room at 21°C. Fifteen days after planting, the canes were dug and split for examination of fungus spread.

Results. — *Sporulation and morphology.* — Large numbers of oospores were observed on oats on which the sterile *Phytophthora* isolate was growing abundantly. When oat grains, covered with the fungus, were placed in dishes with sterile water, many sporangia and chlamydospores were produced within 48 hours (38). Sporangia were even more abundant when the oats were placed on wooden rods in large flasks filled with tap water instead of sterile water. No fruiting structures were ever observed on oatmeal agar or on any other culture medium, except for some chlamydospores in water. This confirms the findings of previous workers (30, 32).

All terms used in the following description and identification of the sugarcane *Phytophthora* isolate are according to the standard terminology of this genus proposed by Blackwell (6).

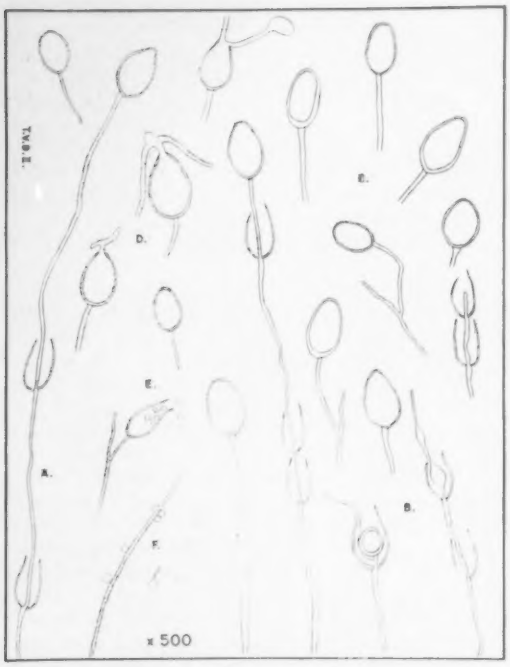

Fig. 1. Camera lucida drawing of sporangia and zoospores of *Phytophthora megasperma* from sugarcane. **A)** Long sporangiophore with proliferation. **B)** Proliferation of nested type. **C)** Sporangia with inconspicuous papillae. **D)** Direct sporangial germination. **E)** Germination by zoospores. **F)** Encysted zoospores.

The young mycelium was typically coenocytic but became septate with age. Hyphae were typical of the genus *Phytophthora:* very variable in diameter, even along the same hypha, and possessing numerous hyphal swellings. The diameter, excluding swellings, was 1.7-5.1 μ.

Sporangia were borne on long sporangiophores (50-200 μ), many measuring over 400 μ long (Fig. 1-A). There was occasional branching of the sporangiophore. An interesting feature was that a single sporangiophore had excessive proliferations of the extended type, producing a new sporangium a short distance ahead of the old, empty one. As many as 6 empty sporangia were observed on 1 sporangiophore. A few proliferations were of the nested type (Fig. 1-B). Sporangiophores of the compound sympodial type, as in *P. infestans* or *P. phaseoli*, were never found nor were those of the simple sympodial type, characteristic of *P. cactorum* (6).

Sporangia were ovoid to bluntly ellipsoid and had inconspicuous papillae (Fig. 1-C). Width was 20-39 μ and length 27-56 μ, averaging 27 x 42 μ. They germinated most frequently by production of zoospores, though direct germination was occasionally observed, the sporangium producing 1 germ tube through the germ pore or opened papilla (Fig. 1-D). No germination with more than 1 germ tube was observed. This differed from reported results with *P. cryptogea* (28), *P. megasperma* (14), and *P. terrestris* (31).

Liberation of zoospores occurred in a manner typical of *Phytophthora*, without the production of an emission tube ending in a vesicle into which the undifferentiated mass of protoplasm is discharged, characteristic of the genus *Pythium* (Fig. 1-E). In a few cases, however, the zoospores stuck momentarily to the mouth of the sporangium in an evanescent cluster, before bursting forth all at once, a condition also described for *P. cactorum* (44). The wall of this cluster is very thin and difficult to see. The number of zoospores from 1 sporangium varied from 12-45. They were irregular in shape at first, but became spherical after a short swarming period and measured 7-10 μ (average 9 μ) upon encystment, which was usually along the hyphae (Fig. 1-F).

Chlamydospores were typically intercalary and with a few exceptions spherical, measuring 20-45 μ (avg. 36 μ). They germinated with 1-7 germ tubes, most commonly with 3-4.

Besides chlamydospores, hyphal swellings were numerous, in some cases closely resembling chlamydospores. They were, however, not cut off from the hyphae by a septum, as were the chlamydospores.

Oogonia, antheridia, and oospores were observed in abundance. Oogonia were spherical, and ranged from 25-41 μ to 26-42 μ, averaging 35 μ. Antheridia were usually paragynously attached to oogonia, but several of these male structures were amphigynous. They were 12-15 μ wide and 12-22 μ long.

Oospores were spherical and plerotic, and measured 18-38 μ to 20-37 μ. They possessed a thick endospore, up to 10 μ wide. Germination of oospores was not observed.

Growth-temperature relations.—The effect of temperature on growth of the sugarcane *Phytophthora* isolate was measured by recording, every other day, the diameter of a colony grown on PDA. These data

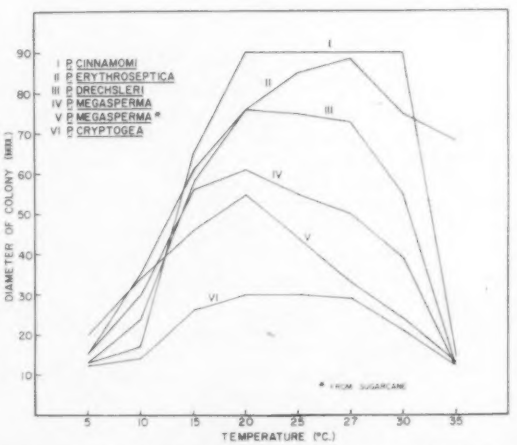

Fig. 2. Comparison of average radial growth of 5 *Phytophthora* species with *P. megasperma* from sugarcane, in plate cultures on PDA after 5-day incubation at 5-35°C.

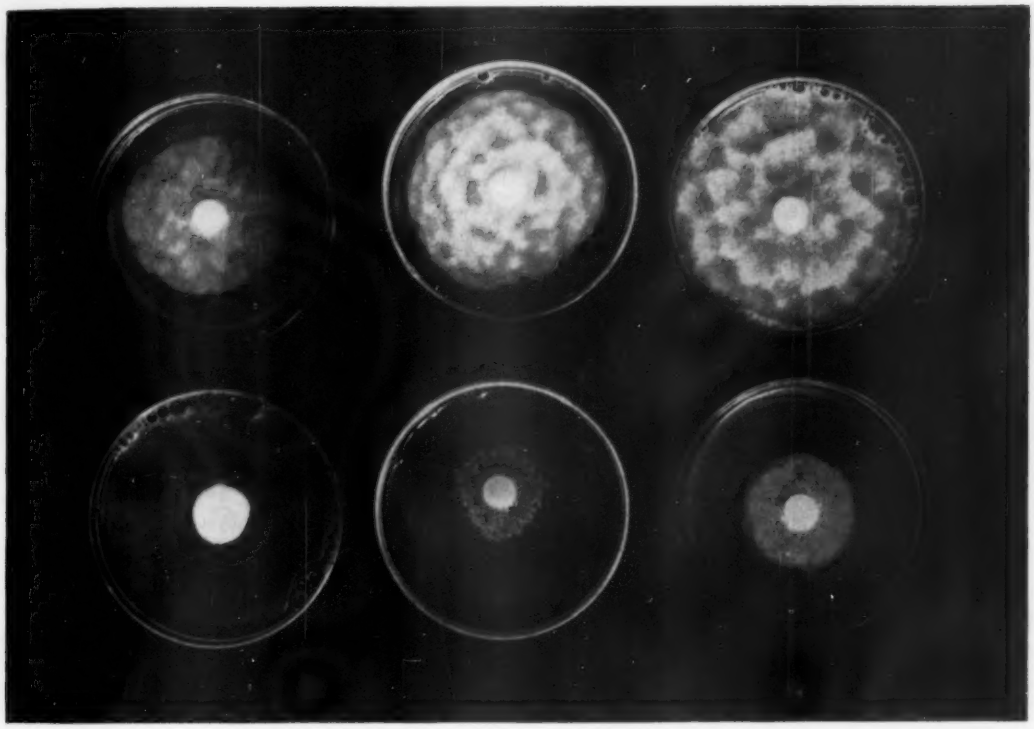

Fig. 3. Average radial growth of 5 *Phytophthora* species and *P. megasperma* from sugarcane, on PDA incubated 5 days at 20°C. Left to right: top, *P. drechsleri*, *P. erythroseptica*, and *P. cinnamomi*; bottom, *P. cryptogea*, *P. megasperma* (from sugarcane), and *P. megasperma*.

were compared with those obtained from other *Phytophthora* spp. tested similarly. After 5 days (Fig. 2), *P. cinnamomi*, *P. erythroseptica*, and *P. drechsleri* had grown faster than the other species at 15-30°C. *P. cryptogea*, on the other hand, made the least growth at all temperatures tested, whereas growth of *P. megasperma* closely paralleled that of the *Phytophthora* isolate from sugarcane, with a little more growth than the latter at 15-30°C.

The optimum temperature for growth of the sugarcane isolate was between 20° and 22°C. In PDA plate cultures, this isolate differed from all other species tested in growing mostly submerged in the agar with very little aerial mycelium (Fig. 3). All species tested had similar minimum and maximum temperatures, except *P. erythroseptica*, which made good growth at 35°C.

These results rather closely confirm those of other investigators. Tucker (37), from temperature studies of *Phytophthora* spp., reported that *P. cinnamomi* grew best at 20-30°C, with an optimum at 25-27°C. Wager (40), who studied 2 strains of *P. cinnamomi*, from avocado in South Africa and from citrus in Brazil, found optima of 25° and 31°C, respectively. For *P. drechsleri* and *P. erythroseptica*, Tucker (37) found growth optima at 27.5-32°C and 27.5°C, respectively, with no growth of the latter species at 35°C. Data on *P. cryptogea* in the present study, however, confirm very closely those reported by Erwin (15) and Middleton, *et al.* (26), who reported minimum temperatures of 8° and 1°C, optima of 25° and 22-25°C, and respective maxima at 30-33° and 31-34°C.

Jones (19) found, in a study on a root rot of sweetclover caused by *P. megasperma*, that the disease was equal in severity over a soil temperature range of 10-24°C. Wager (41) stated that *P. megasperma* from orange roots had an optimum radial growth in culture at 19°C, with a range of 4-28°C. Singh (32), from a study of sugarcane seed-piece rot, reported optimal growth of the sterile *Phytophthora* isolate at 11°C in plate culture.

An interesting feature of this temperature study was that both *P. megasperma* and the sugarcane isolate made fair growth (average diameter 50 mm) after 1 month's incubation at 5°C, compared with growth of 16-34 mm of the other 4 *Phytophthora* spp. At 35°C, only *P. erythroseptica* filled the plates entirely after 30 days, whereas all other cultures barely grew away from their initial inoculum. This shows again the close identity of the sugarcane *Phytophthora* isolate to the type culture of *P. megasperma*; and that this species seems quite capable of survival and growth at

this low temperature and in the range 5-20°C, which conforms with the severity of *Phytophthora* seed-piece rot at low winter temperatures.

The growth-temperature relation of the type cultures of *P. erythroseptica* and *P. megasperma*, in comparison with the sugarcane isolate, was tested on 5 additional culture media at 10°, 20°, and 30°C. The colony diameters were recorded after 6 days. The 2 mineral media, Czapek's and Fries' agar, were unfavorable for the growth of the *Phytophthora* isolates. Oatmeal, cornmeal, and lima-bean agar gave about the same amount of growth as PDA, whereas *P. megasperma* again closely paralleled the growth of the sugarcane isolate on all media used.

Pathogenicity.—With inoculated fruit in moist chambers, both *P. megasperma* and the sugarcane isolate were nonpathogenic to eggplant, and weakly pathogenic to apples; both isolates produced a slow, brown leathery rot in oranges. Govi and Tassinari (16) reported that *P. megasperma*, isolated from peach trees in Italy, also infected apples upon inoculation. Wager (39) found, upon inoculation of oranges and lemons, a type of brown rot similar to that observed in the present study. These comparisons seem to affirm the close relationship of the sugarcane *Phytophthora* isolate to *P. megasperma*.

In pathogenicity tests in which *P. megasperma*, *P. erythroseptica*, and the sugarcane *Phytophthora* isolate were compared for seed-piece rotting ability, the average length of the diseased area in the seed pieces was measured 15 days after inoculation.

The sugarcane isolate was about twice as virulent as *P. erythroseptica*, based on size of diseased internode area. This confirms previous results (32, 34). The type culture of *P. megasperma* appeared more pathogenic than *P. erythroseptica*, but only slightly less so than the sugarcane isolate.

In comparing all 3 isolates on 2 sugarcane varieties, Co. 290 was less susceptible to the *Phytophthora* cultures than was C.P. 34-120. Diseased tissue of C.P. 34-120 had typical pink discoloration, whereas Co. 290 tissue was only water-soaked.

Identification.—For identification of the principal *Phytophthora* spp. responsible for seed-piece rot of sugarcane, a review was made of all known *Phytophthora* spp. (37). Waterhouse's (42) compilation of descriptions and illustrations of all previously recorded species of *Phytophthora* was used.

Of 72 described species, about 20 are known to cause rotting of roots, corms, tubers, or other underground parts. Eliminated were those known to attack specific hosts only, like *P. quininea* on *Cinchona*, *P. primulae* on *Primula*, and *Polyanthus* and *P. richardiae* on Arum Lily. Thus, *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*, and *P. megasperma* resembled most closely the *Phytophthora* isolate from sugarcane.

These species are characterized by having nonpapillate sporangia, and sporangiophores that proliferate by resuming growth through the base of old evacuated sporangia, to produce new ones some distance ahead (37, 42). In addition, *P. megasperma* and *P. cinnamomi* have long sporangiophores, measuring 50-200 μ or more (14, 42). These 3 sporangial characteristics occur in the *Phytophthora* isolate from sugarcane. In addition, *P. erythroseptica* also was considered since this species already had been described from sugarcane as a weak pathogen of seed-piece rot (32, 34).

The width of the sporangia (20-39 μ) of the sugarcane isolate falls in the range given for *P. cinnamomi* (27-39 μ) and *P. megasperma* (6-45 μ). The sporangia of *P. cryptogea* and *P. drechsleri*, in contrast, are more narrow (avg. 21 μ).

P. cinnamomi is distinguished by its typical terminal chlamydospores; those of *P. megasperma* and the sugarcane isolate are only intercalary. Chlamydospores have not been reported from *P. erythroseptica*, *P. drechsleri*, or *P. cryptogea*. The hyphal swellings of *P. cinnamomi* are large spherical bodies in clusters, compared with the smaller single swellings of *P. cryptogea* and *P. erythroseptica*, usually produced in chains (6, 37), also termed sphaero-conidia by Lafferty and Pethybridge (20). In the sugarcane isolate, in contrast, the hyphal swellings are much more isolated, are smaller than those of *P. cinnamomi*, and larger than those of *P. cryptogea*.

Comparing the sexual fruiting structures of these *Phytophthora* spp., *P. erythroseptica*, *P. drechsleri*, and *P. cryptogea* produce only amphigynous antheridia, whereas *P. megasperma* produces both amphigynous and paragynous antheridia. In the original description of this species, Drechsler (14) reported that 35% of the antheridia were amphigynous and the rest paragynous. Paragynous antheridia are usually attached near the oogonium stalk, as in the sugarcane isolate.

Oospores have seldom been observed in *P. cinnamomi*. Ashby (3), however, did find some in old oatmeal agar cultures. *P. erythroseptica*, *P. drechsleri*, and *P. cryptogea* have smaller oogonia (avg. 31 μ) than *P. megasperma*, and consequently smaller oospores (25-30 μ). The sugarcane isolate, however, has oogonia (35 μ) and oospores (31 μ) slightly larger than those of the 3 species just mentioned, but smaller than those of the type species of *P. megasperma*, which respectively measure 42-52 and 37-47 μ. The isolate from sugarcane also resembled the latter species in having a rather thick endospore.

The authors believe therefore that the so-called sterile *Phytophthora* isolate from sugarcane is morphologically distinct from the 4 closely related species mentioned above. Because of 1) its close morphological similarity to *P. megasperma;* 2) almost identical growth in culture at the minimum, optimum, and maximum temperatures; and 3) similar pathogenicity on test fruit and sugarcane seed pieces, the principal *Phytophthora* isolate causing seed-piece rot of sugarcane in Louisiana is identified as a small-spored form of *P. megasperma* Drechs.

Cultures of the sugarcane isolate have been deposited in the American Type Culture Collection, Washington, D.C., and the Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands.

DISCUSSION.—*Phytophthora* seed-piece rot of sugarcane can now be added to the list of plant diseases caused by more than 1 *Phytophthora* sp., like the pink rot disease of potatoes (9, 27), buckeye rot of tomatoes (31, 35), and the shanking disease of tulips (9). This seed-piece rot is caused by the previously reported and slightly pathogenic *P. erythroseptica*, and the more virulent *P. megasperma* identified here.

Certain *Phytophthora* spp. do not produce sporangia or oospores under all conditions or on all culture media. Many investigators have tried numerous methods to induce fruiting, such as different hosts, fruit, soil leachate, sucrose solutions (4, 5, 18, 21, 22, 25). Also, pairing certain cultures of *Phytophthora* has often resulted in the production of sexual organs (2, 12, 33).

Induction of fruiting by using whole oats as culture medium has been known since Clinton (11) in 1910 discovered oospores of *P. infestans*, by using oats or oat juice in different culture media. He found that oogonia were produced quite frequently, and that antheridia fertilized the oospheres only with the addition of oat juice. The oat juice contained lecithin, known to be a constituent of nerve tissue and spermatozoa.

For additional proof of the identity of the sugarcane isolate, cultures were sent to J. T. Barrett in California and G. M. Waterhouse in England. Barrett, from his examination of the isolate, suggested the isolate was *Phytophthora miyabeana* (*Pythiomorpha miyabeana* Ito & Nagai). The genus *Pythiomorpha*, since its erection in 1910, however, has been revised. Buisman (8), in 1927, included *P. gonapodyides* in the genus *Phytophthora* as *Phytophthora gonapodyidea* (Petersen) Buism., while Waterhouse (43) suggested more recently that *Pythiomorpha miyabeana* be placed in synonymy with *Phytophthora megasperma*. She compared a culture of *P. miyabeana* with an isolate of *P. megasperma* described by Tompkins, *et al.* (36) from root rot of cauliflower in California, and also with *Phytophthora* isolates from soybean roots in Ontario and from peach roots in New South Wales. Her conclusion was that the only appreciable difference between the latter 3 pathogens and *P. miyabeana* was smaller oogonia, and consequently smaller oospores.

Tompkins, *et al.* (36) reported from his study of *P. megasperma*, oogonia with a range of 36-52 μ (avg. 43 μ), compared with the type culture identified by Drechsler (avg. 47 μ) (14), whereas *Pythiomorpha miyabeana* had oogonia with an average diameter of 38 μ, in the range 32-46 μ (43). These latter measurements also fit the range and average of oogonia of the sugarcane isolate.

Waterhouse stated that the sugarcane isolate seemed to be a small-spored form of *P. megasperma*. This report confirmed Barrett's identification of the sugarcane isolate, considering *Pythiomorpha miyabeana* synonymous with *Phytophthora megasperma*.

In 1959, Hildebrand (17) reported a root and stalk rot of soybean from Ontario, and described the pathogen as almost identical with *P. megasperma*. He also reported smaller oospores (avg. 30.7 μ) than in the type species described by Drechsler. In pathogenicity tests, his isolate was highly specific for soybeans, and he suggested varietal rank for the species, and named its first variety, *P. megasperma* var. *sojae*.

All measurements and characteristics of the Ontario soybean isolate are identical to those of the Louisiana sugarcane isolate. Additional pathogenicity tests also may prove high specificity of the Louisiana isolate to sugarcane, which might justify varietal rank.

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PHYTOPATHOLOGICAL NOTE

FUNGI ASSOCIATED WITH LARVAE OF SITONA SPP.

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Sitona spp. (*S. hispidula* F. and *S. flavescens* Marsh.) are found in large numbers throughout New England in soils planted to legumes. The larvae are capable of girdling roots (3, 6, 8) and feeding on nodules (8). Injured roots are predisposed to attack by soil-inhabiting and insect-transmitted fungi, resulting in root decay, loss of roots, and deterioration of legume stands.

Previous work (2, 4) has suggested that *Sitona* spp. were involved in the root-rot complex. Only limited work (1, 3, 7), however, has been conducted on fungi associated with *Sitona* spp. The present study was initiated in 1958 to determine: 1) seasonal association of fungi with larvae of different instars, and 2) the pathogenicity of fungi associated with *Sitona* larvae on clover seedlings.

Larvae of different instars were collected from Ladino white clover (*Trifolium repens* L. f. *giganteum* Lagr.-Foss) plots at Dover and Northwood, N. H., during the growing seasons of 1958, 1959, and 1960. Larvae were washed in running tap water, submerged in 70% alcohol for 1 minute, and then sterilized for 2 minutes in mercuric chloride (1:1,000). Larvae were always rinsed 4 times in water prior to plating on potato-dextrose agar. Five larvae were spaced on each agar plate and incubated at room temperatures (75-90°F). Fungus colonies were identified after 8 days' incubation and then discarded. No attempt was

made to identify bacteria recovered. Pathogenicity of fungi was determined on white and red clover seedlings in test tubes (5). Larvae were sprayed with a spore suspension on sterile filter paper and incubated at room temperatures.

FUNGI ASSOCIATED WITH LARVAE.—Numerous fungi were isolated from *Sitona* larvae during a 3-year period (Table 1). *Beauvaria bassiana* (Bals.) Vuill. was isolated most frequently in 1958, while *Fusarium oxysporum* Schlect, emend. Snyd. & Hans. was most prevalent in 1958 and 1960. *B. bassiana* was more commonly isolated in June, 1958, than in September. *F. oxysporum* constituted a higher percentage of isolates in July, 1960, than during the other sampling periods. Other fungi were isolated less frequently and appeared to be of minor importance. Larvae from the 2 locations yielded essentially the same kinds of fungi.

PATHOGENICITY OF FUNGI ON CLOVER SEEDLINGS.—Tests on Ladino white and red clover (*T. pratense* L.) seedlings indicated that *F. oxysporum*, *F. roseum* LK. emend. Snyd. & Hans., *F. solani* (Mart.) Appel & Wr., and *Trichoderma* sp. were pathogenic. The last produced light discoloration of roots. *F. oxysporum* was most pathogenic of the 3 fusaria tested. Most isolates of *F. oxysporum* killed clover seedlings within 10 days. *B. bassiana* and *Metarrhizium anisopliae* (Metsch.) Sorok. were nonpathogenic to clover seedlings.

Although no additional studies were conducted, the apparent association between pathogenic fungi and *Sitona* larvae suggests their possible joint role in inciting root rot under same conditions.

Admittedly, the test-tube method of inoculation is conducted under atypical conditions. It serves, however, as a quantitative method for determining fungus pathogenicity on seedlings. Until more efficient inoculation techniques are developed for testing older plants, the test-tube method serves as a reliable method for determining pathogenicity of root-inhabiting fungi.

TABLE 1. Fungi isolated from larvae of *Sitona* spp. obtained from Ladino white clover plots at Dover and Northwood, N. H., 1958-60[a]

Fungi	Number of isolates			
	1958	1959	1960	Total
Beauvaria bassiana	31	9	8	48
Pencillium spp.	20	5	13	38
Fusarium oxysporum	9	20	49	78
Metarrhizium anisopliae	6	7	0	13
Mucor spp.	4	3	6	13
Trichoderma sp.	3	1	2	6
Rhizopus stolonifer	2	0	3	5
Cladosporium sp.	2	3	1	6
Fusarium roseum	1	0	2	3
F. solani	1	0	1	2
Alternaria sp.	1	1	0	2
Chaetomium sp.	1	11	36	48
Aspergillus spp.	0	2	3	5
Circinella sp.	0	0	1	1
Unidentified spp.	19	16	24	59
Total	100	78	149	327

[a]Number of larvae plated were 327, 264, and 461 in 1958, 1959, and 1960, respectively.

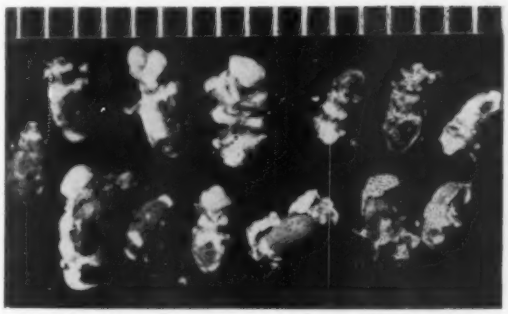

Fig. 1. Adult *Sitona* spp. showing different stages of development of fungi, primarily *Beauvaria bassiana*. Collected from Ladino white clover plots in 1958. (1/16-in. scale.)

PATHOGENICITY OF FUNGI ON INSECTS.—*B. bassiana* and *M. anisopliae* killed larvae 4-8 days after inoculation. Larvae inoculated with fusaria were killed within 10-12 days.

In 1958, adults of both *Sitona* spp. were numerous in clover plots during the late June and July samplings. By August, however, the insect population was very low. Moreover, adults with fungus hyphae growing on both the dorsal and the ventral surface of their abdomens were commonly found on the soil surface of plots (Fig. 1). These fungi were isolated and later identified as *B. bassiana* and *M. anisopliae*.

Jackson (3) suggested that *B. bassiana* was the most serious natural enemy of *Sitona* spp. The facts that *B. bassiana* was isolated most frequently in 1958 and populations were small in late 1958 and early 1959 suggest that biological control helped to reduce the population of *Sitona* in New Hampshire.

This is the first report of entomogenous and plant plant parasitic fungi being isolated from *Sitona* spp. in New England.

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Report and Abstracts of the 37th Annual Meeting of the Southern Division of The American Phytopathological Society

The Southern Division of The American Phytopathological Society held its annual meeting in conjunction with the 58th annual convention of the Association of Southern Agricultural Workers, at Jackson, Miss., Feb. 6-8, 1961. About 90 plant pathologists attended; 36 papers were presented. At the business meeting, February 6, it was announced that the following officers had been elected by mail ballot: President, A. L. Harrison; Vice-President, D. E. Ellis; Councilor, W. J. Martin.

J. P. Fulton, Secretary-Treasurer
Southern Division, A.P.S.

A new strain of sugarcane mosaic virus.—E. V. ABBOTT. —In Louisiana from 1956-59, considerable spread of mosaic occurred in several "resistant" varieties of sugarcane, including the major commercial variety, C.P. 44-101. Apparently, a strain of the virus different than those hitherto recognized was involved. First attempts to identify the strain were unsuccessful because no infection was obtained on the principal differential host variety, C.P. 31-294. This variety is congenial to all the described strains and differentiates 5 of the 7. When inoculations were made to the differential host, C.P. 31-588, infection was readily obtained, and the symptoms differed from those of the described strains. The new strain is designated H. Like strains A and F, it is differentiated from strains B, C, D, E, and G by the absence of leaf necrosis on any differential host variety. It is differentiated from strain A, on C.P. 31-588, by the absence of the discrete, pale yellow to nearly white stripes characteristic of that strain; and from strain F, on C.P. 31-294, by much milder mosaic mottling and extreme stunting of growth. Strain H has been transmitted by artificial inoculation to several forms of *Saccharum spontaneum* hitherto considered immune to mosaic.

Methods of mechanical transmission of bean yellow mosaic virus into white clover.—LUTHER P. ANDERSON and JAMES E. HALPIN.—The transmissibility of Bean Yellow Mosaic Virus (BYMV) from white clover to white clover was tested by: 1) injecting the juice of plants known to be infected by BYMV into either the stolons, tap roots, crowns, or petioles of BYMV-free clover plants; or 2) by dipping the root systems of BYMV-free clover plants into the inoculum. Transmission of the virus by rubbing the leaflets was used as a comparison and control. Symptoms were observed 3 weeks after inoculation. Inoculated plants then were used as a source of plant juice for inoculating garden bean (*Phaseolus vulgaris* var. Bountiful) for verification of virus-transmission. All studies were repeated 4 times using 6 replicates/trial. Injections of BYMV into the stolons gave more pronounced symptoms than rubbing the leaflets, although the frequencies of transmission were equal. Injections into the tap root and crown region were equal in all respects on test plants and controls. Injections into the petioles and dipping the root systems either produced less severe symptoms or reduced frequency of transmission or both. Stolon injections appeared less time consuming than rubbing the leaflets, but may require more inoculum.

Some clovers susceptible to the Cassia, cotton, and other wilt fusaria.—G. M. ARMSTRONG and J. K. ARMSTRONG.—*Trifolium hybridum* (alsike), *T. repens* (White Dutch, Ladino), *T. pratense* (common red, Kenland, Wisconsin Mildew Resistant), *T. incarnatum* (common crimson, Autauga reseeding), and *Melilotus alba* (white sweetclover) were grown in 2-gal pots of sand. Plants 3-9 weeks old were inoculated with wilt fusaria by pouring a liquid culture of the fungus on the cut roots along 1 side of the plant. Inoculations were made with wilt fusaria from alfalfa; bean; *Cassia tora;* cotton, 3 races; cowpea, 3 races; lupine, race B; mimosa, race 1; pea, 2 races; sweet potato, 2 races; and 11 isolates of *F. oxysporum* of unknown pathogenicity from clover. The *Cassia* fungus produced external symptoms of a typical wilt disease in alsike, white sweet, Autauga reseeding, and common crimson clover of 30, 33, 67, and 81% respectively. The U.S. cotton fungus produced external symptoms of wilt on 26% of white sweetclover. No appreciable external symptoms of wilt appeared with other inoculations. Vascular discoloration without external symptoms of wilt was produced in the 4 clovers last mentioned and in 3 varieties of red clover (16-82%) by fusaria from alfalfa, *Cassia*, cowpea, clover, and U.S. cotton.

Observations on white pine needle blights in Tennessee and West Virginia.—C. R. BERRY.—Unusual mortality of white pine has been observed in recent years in east Tennessee. Four plots, each with 40 dominant or codominant trees, were established near Kingston, Tenn., where this condition is prevalent. Four similar plots were established near Marlinton, W. Va., where a similar needle blight was present, but mortality absent. Differences between blights in the 2 areas became apparent from March, 1957 to September, 1960. In West Virginia, a blight of young, developing needles occurred only during the summer (emergence tipburn). In Tennessee, however, trees consistently showed an increase in amount of needle tipburn from July through March (postemergence tipburn). Emergence tipburn is characterized by a sharp margin between the living needle base and the dead tip. With postemergence tipburn, there is an indistinct zone between the living and dead needle tissue, and the base is usually chlorotic and mottled. Ten % of the Tennessee plot trees died after showing postemergence tipburn. In contrast, none of the West Virginia plot trees showing emergence tipburn has died. The West Virginia emergence tipburn is apparently the same as the often reported, widespread "white pine blight." The Tennessee postemergence tipburn is different. Industrial fumes are a suspected cause of the white pine mortality in the Kingston, Tenn., area.

Bacterial blight of poinsettia (Corynebacterium poinsettiae): histopathological studies.—DON B. CREAGER and ENID P. MATHERLY.—Following inoculations of poinsettia plants with pure cultures of *Corynebacterium poinsettiae*, histological studies were made on the path of infection and pathological changes in the tissues. The pathogen was found in substomatal cavities of inoculated leaves, indicating entrance through the stomata. When the pathogen entered through leaf wounds, infection progressed through the vascular tissue and intercellular spaces in the parenchyma of midribs and veins. In the stem, infection progressed from wounds through intercellular spaces in the phloem and cortical parenchyma. Progress was most rapid longitudinally. Occasionally, the pathogen was observed in the xylem vessels. Mostly, however, infection in the stem seemed restricted to the bark area, causing elongated lysigenous cavities as infection progressed.

Mosaic susceptibility of sugarcane seedlings as determined by mechanical inoculation and natural infection.—JACK L. DEAN, OTTO H. COLEMAN, and DEMPSEY M. BROADHEAD.—Mechanical mosaic inoculation of young sugarcane seedlings in the greenhouse is widely used to eliminate susceptible plants in breeding programs. This study investigated possible lack of correlation between the susceptibility of seedlings to mechanical inoculation and their susceptibility to natural infection in the field.

Each sugarcane seedling in the test was split longitudinally as soon as it had produced 2 small tillers and the resulting genetically identical pairs of plants were re-established in the greenhouse. One member of each pair was inoculated mechanically and the other was set into a field interplanted with mosaic infected sorgo. Data were obtained on 815 pairs of plants from 29 crosses.

Apparently, given genotypes of sugarcane seedlings react similarly to natural and artificial inoculation.

Desiccation studies on the soybean cyst nematode, Heterodera glycines, under controlled humidity conditions.—B. Y. ENDO.—Desiccation studies were conducted on cysts, eggs, and larvae of the soybean cyst nematode under 8 relative humidity (RH) levels. Cyst contents (viable eggs or larvae) survived 5 months at 3.2, 8.5, 18.8, 37.1, 58.3, 88.8, and 100% RH. Low survival of cyst contents was noted in cysts stored at 0% RH (concentrated H_2SO_4) after 1 month, but no viable larvae were hatched from eggs in these cysts after 2 months. Viable larvae were recovered from cysts in soil stored at 3.2% RH for 5 months, but not 11 months. Active larvae were found in cysts from all other humidities. In other tests at the same relative humidity levels, viable larvae were recoverd from cysts free of soil stored at 0% RH for 1 month but not 2 months. Viable larvae were found in free cysts stored at 3.2% RH for 1 and 2 months but not 3 months. Active and healthy larvae were recovered from cysts for all other treatments up to 6 months' storage. Eggs on cover glasses in desiccator jars were sampled periodically; viable larvae were recovered up to 4 days' storage at 0, 3.2, and 8.5% RH and up to 8 days at 18.8, 37.1, 58.3, and 88.8% RH. Viable larvae were recovered from eggs stored at 100% RH up to the end of the test period (16 days). Studies on the desiccation of free larvae showed survival at the following maximum lengths of exposure at the various humidities: 6 minutes at 0%; 9 minutes at 3.2%; 10 minutes at 8.5, 18.8, and 37.1%; 15 minutes at 58.3%; 1 hour at 88.8%; and over 1 hour at 100%.

Field trials of new nematocides in 1960 for controlling root-knot nematodes.—J. M. GOOD.—Three application rates of 7 nematocides were compared with DD and unfumigated controls for *Meloidogyne incognita incognita* and *Fusarium* wilt of okra. All but 2 chemicals were chisel injected in the row 10 in. deep and covered with disc-hiller plows. SMDC and DMTT were sprayed into a furrow 4-5 in. deep in a 6-in. band and similarly covered. The most satisfactory application dosage/100 ft of row for each chemical, from the standpoint of nematode and *Fusarium* wilt control, yield increase, and phytotoxicity, was 414 ml of Panogen 161 (methyl isothiocyanate), 258 ml of Panogen 162 (methyl isothiocyanate + dichloropropenes), 1 lb. of 5% G American Cyanamid 18,133 (0,0-diethyl 0-2pyrazinyl phosphorothioate), 140 ml of Pennsalt TD-183 (tetrachlorothiophene), 414 ml of Michigan 1861 (1-bromo-3-chloropropane), 414 ml of SMDC (4 lb/gal), and 1.1 lb of DMTT (85%WP). Panogen 161 and 162 and American Cyanamid 18,133 gave particularly good plant response and nematode control, followed by Michigan 1861 and Pennsalt TD-183. The application method used in this experiment for SMDC and DMTT resulted in less phytotoxicity than has occurred in previous years, but failed to give satisfactory nematode control and yield increases.

Additional evidence on soil transmission of tobacco ringspot virus.—J. W. HENDRIX, J. P. FULTON, and J. L. DALE.—Although soil transmission of tobacco ringspot virus (TRSV) has been demonstrated, the extent of natural soil infestation has not been determined. Results obtained during the summer of 1960 indicate that soil infestation may be a factor in the occurrence of this virus in watermelon plantings. Twelve soil collections were made near plants with melons having blemishes suggestive of TRSV infection. The soil and accompanying melons were collected from 3 widely separated fields in southwest Arkansas. Indexing of the melons indicated that 4 of 12 plants were infected with TRSV. When the soils were potted and planted with watermelons and cucumbers, soil transmission occurred in 4 of the soil collections, 3 of which were obtained near infected watermelon plants. Rates of infection in test plants in these 4 soils were 3-89%. Symptoms appeared in cucumbers and watermelons 18-36 days after planting seed in infested soil.

Basophilia of soil and plant nematodes.—J. P. HOLLIS.—A selective affinity for basic coal-tar dyes in dilute aqueous solutions has been found in species of *Tylenchorhynchus, Aphelenchoides, Tylenchus, Criconemoides,* and juvenile *Rhabditis.* The basophilic reaction is: 1) primarily cuticular, rendering internal structures visible, 2) extremely sensitive with minimum threshold concentrations of 0.1-10.0 ppm, depending primarily on the dye, 3) nontoxic, and 4) reversible. Staining intensity is independent of solution concentration and pH and exhibits a phenomenon resembling metachromasy. Practical significance of the basophilic reaction will depend on the need for rapid differentiation of basophilic nematodes in mixed suspensions and increased optical contrast of cuticular characters for their identification and description.

Gladiolus corm and root rot caused by Penicillium funiculosum.—CURTIS R. JACKSON.—Infection and subsequent necrosis of fibrous and contractile roots of several commercially grown gladiolus varieties in Florida have been caused by the common soil inhabitant, *P. funiculosum.* The fungus also invades vascular tissue of corms by way of root infections. The fungus is weakly pathogenic and root or corm wounds are necessary for infection. Rotting of core and storage tissues of corms occurs most rapidly at 28-32° C. Core rot commonly results in "doughnut" symptoms. Isolates from roots and corm vascular tissues of actively growing plants were more virulent than isolates from corms in cold storage. Greatest mycelial growth of 2 isolates on a V-8 juice-mineral liquid medium occurred at 32°C.

Fusarium wilt of alfalfa in Louisiana.—J. H. JENKINS and G. D. LINDBERG.—A wilt of alfalfa causing a rapid depletion of stands has become a serious problem in the Red River Valley of North Louisiana in the past few years. The roots of diseased plants always exhibit a brown vascular discoloration. Isolations from the discolored vascular tissue and greenhouse inoculation studies have shown that this disease is caused by *Fusarium oxysporum* f. *medicaginis.* Buffalo alfalfa plants, inoculated in sterilized Red River and greenhouse soils, began showing symptoms 20-22 days after inoculation. The leaves turned yellow and the plants suddenly wilted. Occasionally, plants died when only slightly wilted and the leaves retained the major portion of their green color. In some cases, only a portion of the plant wilted and a corresponding portion of the root was infected. Such plants often survived for several weeks. Histological studies showed that many of the vessels were completely filled with the fungus mycelium; others showed vascular plugging, with mycelium difficult to detect.

A leaf spot of cotton caused by Corynespora cassiicola.—J. P. JONES.—Leafspotting of upland cotton *Gossypium hirsutum* was observed at 3 locations in the Mississippi Delta in August, 1959. Varieties affected were Delfos 9169, D. and P., L. 15, and Delfos 8274. Leafspotting also occurred in an experimental nursery on several lines of sea island cotton, *G. barbadense.* The leaf spots were roughly circular with light-brown centers surrounded by narrow dark-brown margins. Diameters of the individual lesions were 2-6 mm. Isolated from the leaf spots was the fungus *Corynespora cassiicola,* the incitant of target spot of soy-

bean and cowpea and blight of sesame. Isolates of *C. cassiicola* from cotton and soybean were cross-inoculated to soybean (Woods Yellow), upland cotton (Delfos 8274, Hartsville, Delta Experiment Station line 7343, and Rex), and sea island cotton (Seabrook). Further cross-inoculations were made with *C. cassiicola* isolates from cotton, soybean, cowpea, and sesame to these respective hosts in all combinations. No differences in pathogenicity were noted among the isolates. The fungus overwintered on infected cotton stems in the field without loss of pathogenicity.

Comparison with a similar cotton leafspotting fungus, *Helminthosporium gossypii*, showed the 2 pathogens to be distinct.

Relative host responses of interplanted weeds and corn to Pratylenchus zeae and P. brachyurus (Nematoda, Tylenchida).—J. M. McBRIDE, D. M. JOHNS, and C. R. CARTER.—Root intermingling of corn and transplanted weeds in a field plot on Shubuta sand provided tests of host preferences and possible antibiosis of weed roots to root lesion nemas in corn. Populations of *Pratylenchus zeae* and *P. brachyurus* were sampled by a modified Young's method in corn and 33 weed species including 22 of the Compositae. Possible hosts, selected on a basis of 67% or 100% of the root samplings yielding nemas, were (*P. zeae*): castorbean, tomato, *Sida rhombifolia, Crotolaria spectabilis, Phaseolus limensis, Aster* sp. and *Helianthus* sp.; (*Pratylenchus zeae* + *P. brachyurus*): *Phlox drummondi, Vinca rosea, Verbena* sp. and *Zinnia* sp.; (No nemas): *Rudbeckia serotina, Brassica napobrassica, Elephantopus carolinianus, Papaver* sp., *Eupatorium* sp., *Tagetes erecta,* and *T. tenuifolia. Pratylenchus* spp. ranged from 30-200/g of roots in corn and 2-14 g in weeds. Ratios of *P. zeae/P. brachyurus* numbers were 18:1 in corn and 10:1 in weeds. Staining tests were not made to detect nemas in the roots. Weeds were nonhosts or at best noncongenial hosts. Data showed there was no antibiosis of weed roots on nema populations in corn.

Studies on the pathogenicity of 4 species of soil fungi on white clover as affected by the presence of bean yellow mosaic virus under conditions of controlled temperature and light.—S. M. McCARTER and J. E. HALPIN.—Pathogenicity of Bean Yellow Mosaic Virus (BYMV) and of 4 species of soil fungi was measured on white clover in growth chambers under uniform light intensity at constant temperatures of 62°, 74°, and 86°F. The virus and each fungus were studied individually and in virus-fungus combinations. Three cuttings of top growth were obtained; then, root and yield observations were made. BYMV reduced the yield of top growth significantly, but did not reduce the amount of root growth or cause significant discoloration of root tissues. *Fusarium oxysporum, F. roseum,* and *Rhizoctonia solani* were the more destructive fungi, alone or in association with BYMV. *Sclerotium batticola* was less destructive than the other 3 species, causing less reduction in yield of tops and roots. All 4 species caused extensive discoloration of clover root tissues. When both a fungus species and BYMV were present, no significant increase in pathogenicity was observed, compared with the corresponding fungus alone. All agents were pathogenic over the entire temperature range tested.

The amino acid and sugar compositions of healthy and anthracnose-infected watermelons.—C. L. McCOMBS and N. N. WINSTEAD.—Fruits of a number of watermelon varieties were harvested at various stages of development, and surface sterilized. Cross-sectional slices or portions were placed in petri plates and inoculated with each of 5 cucurbit anthracnose fungi (*Colletotrichum* spp.). In other instances, the epidermal and subepidermal layers were peeled from mature fruit and similarly inoculated. After 7-14 days' incubation at 25°C, extensive rotting of the inoculated tissues had occurred, regardless of variety. The samples then were extracted with boiling 80% alcohol, the extract lyophilized, and the free amino acids and sugars determined by paper chromatographic techniques. Of the 18 ninhydrin-positive compounds detected in the extracts of healthy tissue, citrulline, and alpha-alanine were predominant. An increase in the glutamic acid and a decrease of glutamine and citrulline contents occurred in the tissue as the disease developed. Glucose and sucrose were the predominant sugars detected in healthy tissue; amounts of these sugars decreased as disease severity increased. Stage of fruit development portion sampled, and isolate used had no detectable qualitative effect on amino acid or sugar compositions.

Root histopathology of root-knot resistant and susceptible cotton.—N. A. MINTON.—Histopathological studies were conducted on primary roots of root-knot resistant and susceptible cotton fixed 2, 7, 14, 21, 29, and 36 days following inoculation with *Meloidogyne incognita acrita* larvae. Resistance could not be attributed to plant morphological differences or to failure of nematodes to enter roots of resistant plants. Roots from the highly resistant plant, a wild selection of *Gossypium barbadense,* were more hypersensitive to nematodes than were 2 varieties of *G. hirsutum* (Auburn 56, moderately resistant; and Rowden, highly susceptible). Hypersensitivity resulted in tissue necrosis and the nematodes thus were unable to complete their life cycle. Nematode-induced hyperplasia and hypertrophy were less severe in *G. barbadense.* Nematodes developing in the roots of *G. barbadense* were usually oriented parallel to and within the stele. In the 2 varieties of *G. hirsutum,* they were located more often outside and at an angle to the stele. Confinement to the stele appeared related to resistance since the nematodes developed more slowly and fewer matured. Possibly, their being located within the vascular cylinder would delay escape of hatched larvae and thus retard secondary infection.

Comparative morphology and respiration of normal and diseased Helminthosporium victoriae.—E. E. PSARROS and G. D. LINDBERG.—A comparative morphological cytological study of normal and diseased cultures of *Helminthosporium victoriae* revealed certain irregularities that characterized the diseased colonies. Young hyphae from newly diseased colonies showed various degrees of lytic degeneration and attempts to detect nuclei in such hyphae were unsuccessful. Similar to normal *H. victoriae,* young hyphae from diseased colonies obtained by subculturing from severely diseased mycelium did not show any signs of lytic degeneration and contained abundant nuclei. These hyphae did, however, show various kinds of distortions in the morphology of cells and in the pattern of growth. In comparative respiratory studies, diseased *H. victoriae* from 10-day-old stationary and from 2- to 6-day-old shake-cultures showed an increased respiratory rate compared with normal fungus. In addition, diseased *H. victoriae* from 4-day-old shake-cultures did not show any increase in respiratory rate in the presence of low concentrations of DNP (5×10^{-5} and $1\times10^{-4}M$), whereas the normal fungus showed a positive response.

Control of pathogenic fungi, nematodes, and weeds by preplanting chemical treatments.—H. W. RANKIN.—Certain soil chemicals were used as preplanting applications 8-10 weeks before planting, followed by throwing a 16-18-in. bed on the treated rows. Due to seasonal low temperatures, the soil within the beds was acted on by chemicals for an extended period, several weeks longer than customary for spring application of soil fumigants. The following treatments were used: PCNB (75% wettable powder), DMTT (50% dust), SMDC (4 lb/gal), CBP (57% by vol), DD, formaldehyde (37% by vol), SD-4741 (an experimental organic sulfur-phosphorus compound, 6.5 lb/gal), and untreated checks. A few days before planting, the tops of the row-beds were removed, exposing a chemically treated strip in which tomatoes were planted. Soil was not moved into the treated band during cultivation.

Data were obtained on the effectiveness of these treatments as herbicides, soil fungicides, and nematocides. DMTT and SMCD gave good control of grass and broadleaf weed seedlings with the exception of some legumes

(beggar weed, crotalaria, etc.), excellent control of *Sclerotium rolfsii*, and fair control of *Meloidogyne incognita*. PCNB was effective as a soil fungicide, but was somewhat phytotoxic. DD and CBP were effective only as nematocides. The other materials were not effective.

Late symptom expression in strawberry-virus indexing.—H. E. REED and E. L. FELIX.—Virus symptoms usually appear 4-6 weeks after excised-leaf grafting on *Fragaria vesca* (EMS). Symptoms appeared, however, 6.0-6.5 months after grafting supposedly virus-free Sparkle. Only leaves in 16 of 24 plants, grafted on May 30 and December 31, 1959, were decidedly narrowed, dwarfed, and deeply serrated or divided. In a study of pepper virus diseases, young leaves of California Wonder were rubbed with diluted juice from the virus infected indicator plants. Large chlorotic areas and upward rolling of the leaves, very similar to the symptoms described for tobacco mosaic, appeared on the inoculated peppers after about 3 weeks. A yellow variant, similar to that found in TMV, also was obtained. Immunological reaction on pepper with this strawberry virus against tobacco mosaic appeared negative, indicating nonrelationship of the 2 viruses. Moreover, the tobacco mosaic virus on inoculation failed to produce symptoms on previously uninoculated *F. vesca*. Pronounced filiformity appeared on some of the inoculated pepper plants 4.5-6.0 months later, suggesting that these symptoms may have been due to the same virus causing filiformity in *F. vesca*; and the earlier symptoms in pepper, by a component of a virus complex.

The efficacy of postplanting applications of DBCP for control of Tylenchorhynchus claytoni in southern forest nurseries.—S. J. ROWAN and J. M. GOOD.—Effectiveness of DBCP (50% by vol emulsifiable 1,2-dibromo-3-chloropropane) for control of stunt nematodes in loblolly pine seedbeds was evaluated as a postplanting drench in 2 experiments in 1959. All treatments were applied in a water solution equivalent to 870 gal of the solution/A and sealed with an additional 0.25 in. of water by overhead sprinklers. Spring applications were applied 61 days after planting at 0.2, 0.4, 0.7, 1.4, 2.9, and 5.7 gal of technical material/A. Fall applications were applied 133 days after planting at 0.0, 4.0, 6.0, 8.0, and 10.0 gal of technical material/A. In the spring test, only the highest dosage (5.7 gal) gave appreciable nematode control. In the fall experiment, fair to good control was achieved for all dosages, but economical control was obtained only at 6 and 8 gal/A. Root development was improved by treatments in excess of 4 gal/A. A small and insignificant increase in seedling height occurred for dosages of 4 and 6 gal/A. Phytotoxicity was evident from the 8- and 10-gal treatments.

Etiology of radish black spot.—R. H. SEGALL.— Black spot has become an important postharvest problem in Florida since radishes have been packed and shipped in plastic bags. It is characterized by brown to black circular lesions 1-3 mm in diameter on the radish hypocotyl. A bacterium isolated from these lesions produced typical symptoms when reinoculated. It was reisolated. Leaf and root spotting by this bacterium was induced on radish (*Raphanus sativus*) and turnip (*Brassica rapa*); and leaf spotting on cabbage (*Brassica oleracea* var. *capitata*), kale (*B. oleracea* var. *acephala*), brussel sprouts (*B. oleracea* var. *gemmifera*), cauliflower (*B. oleracea* var. *botrytis*), mustard (*B. juncea*), tomato (*Lycopersicum esculentum*), and bell pepper (*Capsicum frutescens*). This host range and the morphological, cultural, and physiological characteristics are identical to a described variety of *Xanthomonas vesicatoria* (Doidge) Dowson causing a leaf spot of turnip and radish leaves. This same binomial is suggested as the causal agent of radish black spot. Inoculation and infection occur during the mechanical harvesting and packing operations. Following infection, a 4-day incubation period at 70°F is required for symptom expression.

Potato seed-piece treatment with various chemicals.—J. B. SINCLAIR.—Potato seed-pieces of Red LaSoda were dusted with or dipped in 6.5% zineb; 7.6% ziram; 5% Dyrene (2,4-dichloro-6-0-chloroanilino-3-triazine); 5% each of PCNB plus captan, 100 ppm streptomycin (streptomycin nitrate), 0.83% Agri-mycin-500 (1.76% streptomycin, 42.40% basic copper sulfate and 0.18% oxytetracycline); and various concentrations of potassium gibberellate (0-100 ppm). Stand counts and yields of marketable tubers and culls were taken from randomized, replicated field plots. The results of 4 years' experiments are given. In the absence of seed-piece rot, ziram and PCNB plus captan showed no significant increase in stand or yield over the check; zineb showed some increase only in the first year. Dyrene gave a significant increase in stand and a slight increase in yield, but the latter was not significant. Streptomycin and potassium gibberellate were phytotoxic. Where streptomycin was used, stand and yield were significantly reduced below nontreated checks. Potassium gibberellate, regardless of concentration or whether used as a dust or liquid, reduced stand and yield significantly below checks in all trials.

Semicarbazone of cycloheximide for controlling brown spot needle blight.—G. A. SNOW.—To evaluate its effect on *Scirrhia acicola*, the semicarbazone derivative of cycloheximide, at 25 ppm in water, was sprayed on 50 longleaf pine seedlings of natural reproduction. The current year's needles were about half their mature length and were just beginning to develop brown spot symptoms. Three months after spraying, the proportion of current year's leaf surface with brown spot averaged 8.5%, compared with 43% for unsprayed controls. Twenty isolations of *S. acicola* were obtained from 250 attempts with treated needles, and 85 from the same number of trials with unsprayed needles. These results warrant further tests of the material.

Effect of treating the soil with Bromomethane on yield of sugarcane infected with the ratoon stunting disease virus.—R. J. STEIB, W. BIRCHFIELD, J. P. HOLLIS, and S. J. P. CHILTON.—The ratoon stunting disease (RSD) of sugarcane is caused by a mechanically transmitted virus with no known insect vector. In field and greenhouse tests, Bromomethane (BM) was used to fumigate the soil prior to planting sugarcane, infected with the RSD virus. Disease-free cane was planted as the check. Dichloropropene-dichloropropane mixture (DD) used in 1 field test gave no increase in growth or yield. BM was used at the rate of 2 lb/100 ft². RSD cane planted in BM treated soil gave an increase of 6.1 T/A over RSD cane planted in nontreated soil. RSD-free-cane grown in BM treated and nontreated soil produced relatively the same yield. Similar results were observed in a greenhouse test. Effects of the disease were not completely overcome by the treatment; however, the yield difference between the treated and nontreated RSD plots was considered significant. RSD symptoms were found in diseased cane grown in both treated and nontreated plots. The nematode population was not sufficiently different during the growing season to explain the response obtained when RSD virus-infected cane was grown in soil treated with BM.

Utilization of the photoperiod response in selecting kenaf resistant to root-knot nematodes.—T. E. SUMMERS, F. D. WILSON, and J. F. JOYNER.—The ability of kenaf (*Hibiscus cannabinus* L.) to produce seed under heavy attack by the root-knot nematode (*Meloidogyne incognita acrita*) is an efficient and reliable indication of resistance, more so than gall formation on seedling roots. Plants grown in the greenhouse in heavily infested soil under reduced day-length were quickly forced into flowering and fruiting. Roots were examined, selections made, and seed harvested, all in a relatively short period of time. Approximately 20 times more susceptible plants were eliminated from a given population by this method than by selecting in the seedling stage.

In an early generation yield test of material developed in the manner described, 4 selections of 14 tested yielded 39.56, 28.88, 19.59, and 19.08% more dry fiber than var. Everglades 41; and 19.75, 10.56, 2.62, and 2.18% more dry

fiber, than var. Everglades 71, when grown in heavily infested field soil.

A new disease of swamp tupelo.—E. R. TOOLE.—A new trunk lesion of swamp tupelo (*Nyssa sylvatica* var. *biflora*) was found in several stands in south-central Louisiana in 1959. The lesions result from the killing of limited patches of cambium in successive years in the same general stem area. This results in roughened bark and swellings as external evidences on the stem. The killed cambium patches heal and become overgrown, leaving defects scattered through the wood. These defects will cause considerable degrade of the otherwise high-grade tupelo. *Fusarium solani* was consistently isolated from the active lesions and produced small cankers on 1-year-old tupelo seedlings on inoculation. These results suggest that the lesions are caused by *F. solani*.

Spread of Cronartium fusiforme branch infections.—A. F. VERRALL.—Measurements were made on 8 young pine stands to determine how far branch infection of *Cronartium fusiforme* spread into stems. The resulting stem infections either kill or cause degrade. Of infections originating 0.1-5, 5.1-10, 10.1-15, or over 15 in. from the stem, the percentages reaching the stem during the 1st 18-23 years of stand history were 67, 29, 8, and 0, respectively for slash pine, and 83, 60, 25, and trace for loblolly. Annual proximal growth of 1-3-year-old galls varied from 0-8 in. for each species, but averaged 2.4 $\pm$ 0.19 in. for loblolly and 1.8 $\pm$ 0.13 in. for slash. Growth rates of individual galls varied greatly from year to year. Seventy-three % of all stem galls originated as branch infections. Most infections starting more than 10 in. from the stem were stopped by death of the branch before the fungus could reach the stem. These data justify current recommendations that only branches with galls 15 in. or less from the stem are worth removing in control prunings.

Results of chemical drenches in the control of spring dead spot of Bermuda grass.—D. F. WADSWORTH.—Spring dead spot is prevalent in a number of states where Bermuda grass is an important turf. The disease appears more severe in northern regions where the host is adapted and undergoes a long dormant period; it is less severe as the length of host dormancy decreases. The dead plants have a black, rotted root and rhizome system. Although many fungi, principally *Helminthosporium* spp., have been isolated from dead plants, experiments to reproduce the disease have been unsuccessful. A study of control by use of chemical drenches was started in 1958. The following chemicals were applied in sufficient water to wet the soil to a depth of 3-5 in.: Sulforon (wettable sulfur, 97%), Parzate (zineb, 65%), liquid Dieldrin (dieldrin, 1.5 E.), PMAS (phenyl mercuric acetate, 10%), Elgetol (sodium dinitro-ortho-cresol, 19%, plus sodium butyl naphthalene sulfonate, 5%, plus sodium chromate, 2%), and Actidione R-Z (cycloheximide, 1.3 %, plus pentachloronitrobenzene, 75%). The treatments, with 1 exception, were made at approximately 2-week intervals from late August to early December in 3 replicates. Evaluation of the treatments was made the following May. Satisfactory control was obtained only in those plots receiving Dieldrin.

A premature dying of soybeans in Arkansas.—H. J. WALTERS.—Premature killing of plants of early maturing soybean varieties in sandy type soils has been observed for several years. During late summer, following the stress of short droughts, tops of plants were killed and reddish nodal cankers appeared along with internal reddening of stems. Fungi most frequently isolated from affected plants were *Fusarium* spp., *Diaporthe phaseolorum* var. *sojae*, *Glomerella glycines*, *Sclerotium rolfsii*, and *Macrophomina phaseoli*. Other organisms isolated were *Alternaria* sp., *Cladosporium* sp., *Cercospora* spp., *Curvularia* sp., *Penicillium* spp., *Aspergillus* spp., *Acrostalagmus* sp., *Colletotrichum* sp., *Epicoccum* sp., *Helminthosporium* spp., and several unidentified fungi and bacteria. Symptoms resembling those observed in nature were produced only by inoculation with *M. phaseoli*. Pod mottle and tobacco ringspot viruses were isolated from affected plants; the latter virus produced symptoms similar to premature killing. Fumigation with methyl bromide significantly increased yields and reduced prevalence of *M. phaseoli*. Premature killing seems caused by an interaction of several factors; *M. phaseoli* and possibly tobacco ringspot virus may be important contributing factors.

Permeability changes caused by victorin.—H. WHEELER.—Victorin, a specific toxin produced by *Biopolaris* (*Helminthosporium*) *victoriae*, induces symptoms typical of Victoria blight of oats. Since permeability changes often occur in diseased plants, the effect of victorin on cell permeability was studied. Cut stems of resistant and susceptible oats were placed in solutions of crude victorin, in concentrations of 10^{-1} - 10^{-4}. After 2 hours' uptake, 1-g samples were washed and placed in flasks containing 100 ml of distilled water. Changes in permeability were estimated from changes in electrical conductivity of the ambient solutions. For lower concentrations of toxin, 10^{-5} - 10^{-9}, the washed tissues were placed in solutions of toxin adjusted to a constant level of conductivity with NaCl. Control solutions of deactivated victorin caused no significant changes in permeability at any concentration tested. With resistant tissues, the same was true for all active victorin solutions. With susceptible tissues, victorin caused marked increases in permeability and the magnitude of the effect was proportional to the concentration of toxin. The lowest concentration which produced a response was 10^{-7}. Since the crude victorin had an end point of 5×10^{-5} in the root growth test, permeability changes may provide a more sensitive bioassay for this toxin than root inhibition.

Dwarf fascicle abnormality of Pinus echinata Mill.—P. A. YOUNG.—Three-year-old short leaf pine trees in August 1959 showed few to many fascicles on which all or nearly all of the needles were only 0.33 - 1.0 - in. long in contrast to normal lengths of 3-5 in. When most of their fascicles were dwarfed, the trees were about half normal size. The dwarfed needles were abnormally bluish green when young, but purplish, reddish, or yellowish-green when old. The trees showed some dead roots. Moderate numbers of dagger nematodes (*Xiphinema americana* Cobb) occurred in surrounding soil. Many abnormal trees showed needles on the bases of their trunks. Abnormal trees were treated with Nemagon (5 gal/A) but showed no effects from the Nemagon 6 months later. The abnormal trees receiving 2 lb of 16-20-0 or 13-13-13 fertilizer/tree showed no effect from the fertilizer 9 months later. Specimens of the abnormal twigs, sent to forest pathologists, were not identified as having a known disease. The cause was not determined. The symptoms indicate a virus disease like yellows. *Rumex hastatulus* Baldw. and *Erigeron canadensis* L. commonly show aster yellows near the abnormal pines.

Significance of ascigerous stage of Mycosphaerella aleuritidis in primary infection of tung in 1960.—T. VAN DER ZWET.—Leaf spot of tung (*Aleurites fordii*) is caused by *Mycosphaerella aleuritidis;* the imperfect stage is a *Cercospora* sp. In 1960, infection was observed in mid-April on leaves of 1- to 2-year-old volunteer seedlings, growing from unharvested seed and in mid-May on leaves of mature trees. The latter leaves appeared 3 weeks later than those on the volunteers. At the end of June, infection was observed on leaves of new volunteers from 1959 seed. These leaves appeared about the end of May. Incidence of the disease was greater on volunteers in the tree row, where old leaves covered the ground, than on those in the middle, where leaves had been destroyed by cultivation. Incidence was greater also on 1- to 2-ft volunteers than on taller ones. Since the earliest infections on volunteers did not produce conidia until about July 1, primary infection may have been caused mainly by ascospores discharged between April 1 and June 1 from the old leaves. Dissemination of the ascospores may be somewhat limited, vertically and horizontally. The fact that infection occurred later and to a lesser extent in an isolated orchard, where practically all of the old leaves had been destroyed, supports this conclusion.

Bibliography of Reviews

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Monographs and Reviews

TABLE OF CONTENTS

INTRODUCTION 648

GENERAL CONSIDERATIONS 648
- Historical and Related Material 648
- Bibliographies, Catalogs, Lists, Maps 649
- Disease Identification and Evaluation 649
- Disease Impact 649

CAUSAL FACTORS 650
- General 650
- Nonpathogenic Factors 650
- Pathogenic Factors 650

HOST PLANTS 652
- Natural Communities 652
- Ornamentals 652
- Cultivated Crops 652
- Individual Species and Varieties 652

HOST-PATHOGEN INTERACTION 653
- Disposition of the Host; Vulnerability 653
- Entry of Pathogen 653
- Disease Establishment and Spread 653

DISEASE IN POPULATIONS 654
- Inoculum 654
- Epidemic Development 654
- Environmental Factors 655
- Role of Insects 655

DISEASE CATEGORIES 655
- Types 655
- Causal Agents 656
- Individual Diseases 656

DISEASE CONTROL 658
- Economic Considerations; General 658
- Chemical Control 658
- Disease-Resistant Varieties 659
- Quarantines 660
- Cultural Control 660
- Special Problems 660

INSTRUMENTATION; RESEARCH TECHNIQUES 661

ADDITIONAL JOURNALS CITED 661

INTRODUCTION

Prepared under supervision of the Committee on Phytopathological Monographs and Reviews. The costs of bibliographic and clerical work for this project were met from funds provided by the National Science Foundation; expenses of publication were borne by The American Phytopathological Society.

Continuing the program of the past few years, the literature available in plant pathology was surveyed in early 1961 for reviews appearing since the publication of the initial compilation (Part 2, Number 8, of *Phytopathology*, August, 1960). Practically all of this work was carried on through the facilities of the U.S. Department of Agriculture library, Washington, D.C.

The 250 citations noted below do not all, of course, come from journals and other sources dated 1960. Some are dated earlier, but have become available only during 1960; a few, dated 1961, appeared on the shelves during the January-March period of the survey. The net result is, then, an annual supplement centering about, but not confined to, the year stated in the title.

The procedure outlined in some detail in the introduction to the 10-year initial bibliography has been followed here with but minor deviations. Again, the classification is handled in such a way that a given item appears only once, despite the fact that it might appropriately be placed in several categories. Because there are only about a tenth as many items to handle in the supplement, the smallest categories have been omitted—reference to the Table of Contents of the initial 10-year survey will indicate the lesser subdivisions then employed.

Citations are, again, as nearly as possible those of *Chemical Abstracts*. A list of journal titles, including only those not published in 1960, is appended to this supplement.

GENERAL CONSIDERATIONS

Historical and Related Material

Diercks, R. 1959. Pflanzenschutzliche Versuchs- und Forschungs-Tätigkeit der Bayerischen Landesanstalt für Pflanzenbau und Pflanzenschutz, 1948-1958 [Experimental and research work on plant protection at the Bavarian State Station for Plant Cultivation and Plant Protection, 1948-1958]. *Pflanzenschutz* 11:2-14.

Virus diseases; fungus diseases; nutrition, metabolism, and the tendency to disease; diseases and injuries of nonparasitic origin; nematodes; weed control; biological pest control. 233 refs.

Horsfall, J. G., and A. E. Dimond, eds. 1960. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. 715 p.

The pathogen: the concept of causality. The nature, origin, and evolution of parasitism. The multiplication of viruses. Reproduction of bacteria, actinomycetes, and fungi. Spore germination. The mechanical ability to breach the host barriers. Chemical ability to breach the host barriers. Interaction of pathogen, soil, other microorganisms in the soil and host. Toxins. Heterokaryosis, saltation, and adaptation. Genetics of pathogenicity. Virus inactivation *in vitro* and *in vivo*. Physiology of fungitoxicity. Fungicidal chemistry. Nematocides. Author index. Subject index.

Horsfall, J. G., and A. E. Dimond, eds. 1960. Plant pathology. Vol. 3: The diseased population; epidemics and control. Academic Press, New York and London. 675 p.

Inoculum and the diseased population. Inoculum potential. Autonomous dispersal. Dispersal of inoculum by insects and other animals, including man. Dispersal by air and water—the take-off. Dispersal by air and water—the flight and landing. Analysis of epidemics. Forecasting epidemics. Quarantines. Cultural practices in disease control. Soil treatment. Performance of fungicides on plants and in soil—physical, chemical, and biological considerations. Biological interference with epidemics. The problem of breeding resistant varieties. Author index. Subject index. Total of 1,264 refs.

Krause, J., ed. 1957. Proceedings of the IV International Congress of Crop Protection. Vol. 1. Bibliothek der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Braunschweig. 1,048 p.

This first of 2 volumes contains sections on: general plant protection; microbial and nonparasitic diseases; warning services and forecasting; viruses and virus diseases; weeds; plant pathogenic nematodes; mites; noxious insects; biocoenoses; and biological control. Within each section are numerous papers.

Levine, M. N., and Rita Basile. 1960. A review and appraisal of thirty years' research on cereal uredinology in Italy. *Boll. Staz. Patol. Vegetale*, Series 3, 17(1959):1-32.

Parasitic races isolated and identified by Italian workers; relative occurrence of parasitic races isolated in countries concerned; definitions of uredinological research terms; classification of reaction and

infection differentiations; consolidation of parasitic races and simplification of diagnostic keys; formula for estimating greatest number of rust races possible. Italian summary. 36 refs.

Mühle, E. 1958-59. Forschungsarbeiten des Institutes für Phytopathologie der Karl-Marx-Universität [Research programs of the Institute for Phytopathology of the Karl-Marx-University]. *Wiss. Z. Karl-Marx-Univ. Leipzig* 8:707-749.

Studies on diseases and injuries of forage plants and of drug and aromatic plants; potato viruses and their vectors. References, totaling 118, follow each topic.

Stakman, E. C. 1959. The need for international cooperation on cereal rusts. *In* Omagiu lui Traian Săvulescu cu prilejul implinirii a 70 de ani [Honoring Traian Savulescu on the occasion of his 70th birthday]. Editura Academiei Republicii Populare Romîne, Bucharest. p. 745-753.

Review of cereal rusts and control efforts. Rumanian summary. 17 refs.

Zanardi, D. 1959. Un cinquantennio di prove di lotta contro la "Moria" del peperone [Fifty years of tests on the control of "dieback" of Capsicum]. *Notiz. Malattie Piante* 49-50:18-28.

History of the control measures adopted by various workers in Italy since 1907. English summary. 20 refs.

Bibliographies, Catalogs, Lists, Maps

Bärner, J., comp. 1959. Bibliographie der Pflanzenschutzliteratur 1952 [Bibliography of plant protection 1952]. Paul Parey, Berlin. 433 p.

More than 12,700 titles. Introduction, table of contents, and section headings in German, English, and French. Author index.

Bärner, J., comp. 1960. Bibliographie der Pflanzenschutzliteratur 1953 [Bibliography of plant protection 1953]. Paul Parey, Berlin. 527 p.

Approximately 15,000 titles. Introduction, table of contents, and section headings in German, English, and French. Author index.

Boalch, D. H., ed. 1960. World directory of agricultural libraries and documentation centres. International Association of Agricultural Librarians and Documentalists, Harpenden, Herts. 280 p.

Two thousand five hundred and thirty-one entries, listed geographically. Subject index in English, with cross-references from selected agricultural terms in French, German, Russian, and Spanish, and limited to libraries specializing in not more than 2 subjects (includes plant pathology and plant protection). Alphabetical place index listing countries, states, and towns.

Gonçalves, C. R. 1957; 1959. Resenha bibliográfica fitossanitária Brasileira [Bibliographical review of Brazilian literature on plant protection]. *Bol. Fitossanit.* 7:83-141; 8:63-141.

Annotated citations, arranged under entomology, phytopathology, insecticides and fungicides, and miscellaneous.

Rosa, M. 1959. Contributo ad una bibliografia fitopatologica italiana per l'anno 1958 [Contribution to a bibliography of plant pathology in Italy for the year 1958]. *Boll. Staz. Patol. Vegetale* 16:211-233.

Contributions to the literature of phytopathology appearing in Italian journals during 1958. Arranged alphabetically by author. 475 titles.

Disease Identification and Evaluation

Garber, K. 1960. Erfahrungen mit der Blattanalyse bei Rauchschadenuntersuchungen [Experiences with leaf analysis in smoke injury investigations]. *Angew. Botan.* 34:33-37.

Points out certain conditions in which microchemical qualitative analysis of leaves can be helpful in diagnosing smoke injury. 12 refs.

Disease Impact

Holmes, E. 1960. The contribution of crop protection to increased world food supplies. *Outlook on Agr.* 3:23-37.

General review of the advances of the past 2 decades. 39 refs.

Miller, P. R. 1960. New techniques for plant disease surveys and for appraisal of losses. *Plant Disease Reptr.* 44:5-11.

Advances in survey methods; investigations on the spread of pathogens; assessment of importance of plant diseases. 20 refs. Annotated supplementary bibliography of 44 titles.

Strickland, A. H. 1960. Pest and disease losses in agriculture. *New Scientist* 8:660-662.

General review of the world situation: use of crop loss estimates; estimation of losses; some examples.

CAUSAL FACTORS

General

Horsfall, J. G., and A. E. Dimond. 1960. The pathogen: the concept of causality. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 1-18.

The meaning of pathogen and pathogenesis; the kinds of pathogens; proving pathogenicity; the coexistence problem in disease; the long struggle to establish causality. The organization of Volume 2. 18 refs.

McNew, G. L. 1960. The nature, origin, and evolution of parasitism. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 19-69.

Interrelationship of parasitism and pathogenicity; the physiology of pathogenicity; the natural processes of pathogenesis; the evolution of parasitism and pathogenicity; the law of host-parasite balance in pathogenesis; the effect of environment on different classes of parasitism; host genetics in relation to type of pathogenicity; the search for effective disease control by application of logical principles. 76 refs.

Nonpathogenic Factors

Bruce, R. S. 1955-56. A study of the factors influencing the selective action of phytotoxic compounds. Doctoral dissertation, St. John's College, Oxford University. GBI 6.

Daines, R. H., Ida A. Leone, and Eileen Brennan. 1960. Air pollution as it affects agriculture in New Jersey. *Hort. News* 41:3487-3491.

Investigations on the effects of sulfur dioxide, hydrogen fluoride, chlorine, 2,4-D herbicides, and oxidants. 28 refs.

Dinkel, D. H. 1960. A study of factors influencing the development of hollow heart in Irish cobbler potatoes (Solanum tuberosum L.). Doctoral dissertation, University of Minnesota. DA 21. 84 p.

Garber, K. 1960. Neuere Literatur über Rauch-, Staub- und Abgasschäden [Recent literature on smoke, dust and gas injury]. *Angew. Botan.* 34:65-103.

Effect of specific gases, smog, coal and other dusts, and radioactive air pollution. 331 refs.

Guderian, R., H. van Haut, and H. Stratmann. 1960. Probleme der Erfassung und Beurteilung von Wirkungen gasförmiger Luftverunreinigungen auf die Vegetation [Problems of detection and assessment of the effects of gaseous contaminants in the air on vegetation]. *Z. Pflanzenkrankh. u. Pflanzenschutz* 67:257-264.

The concept of "damage"; methods for determination of injury; avoidance of injury. English summary. 15 refs.

Henzell, E. F. 1955-56. Some aspects of the physiological action of phytotoxic compounds. Doctoral dissertation, St. Edmund Hall, Oxford University. GBI 6.

Jacobius, A. J., J. R. Gibson, V. S. Wright, Wave E. Culver, and Ludmilla Kassianoff. 1959. The air pollution bibliography. Vol. II. The Library of Congress, Science and Technology Division, Bibliography Section. 176 p.

One thousand five hundred and fifty titles, alphabetized by author; secondary author index; subject index. Lists items under chlorosis, citrus plants, crops, ferns, flowers, forests, plant leaves, trees, vegetation.

Mulder, D., and R. L. de Silva. 1959. Deficiency diseases and the symptoms of magnesium deficiency. *Tea Quart.* 30:157-165.

Types of deficiency symptoms; influence of weather conditions on symptom development; literature review; results of the authors' investigations, 7 refs.

Scurfield, G. 1960. Air pollution and tree growth. I and II. *Forestry Abstr.* 21:339-347; 517-528.

Origins and effects of air pollution; diagnosis; physiological and meteorological aspects; mitigation and prevention. Bibliography of 242 titles.

Weber, Anna. 1960. Molybdaenmangel [Molybdenum deficiency]. *Gärtner-Tidende* 76:192-195; 204-206.

Summary of available information based on a comprehensive survey of the literature, with special reference to molybdenum deficiency in cauliflower in Denmark and elsewhere. The author explains that the literature covered is too extensive to be listed, and indicates where the numbered references may be found.

Pathogenic Factors

Agatov, P. A. 1958. [Role of chemically active groups of the tobacco mosaic virus and of its virulent activity.] *Trudy Inst. Microbiol., Akad. Nauk SSSR* 5:265-281. (In Russian, 74 refs.)

Bawden, F. C. 1959. Viruses: retrospect and prospect. The Leeuwenhoek Lecture. *Proc. Roy. Soc. (London)*, Series B, 151:157-168.

Present knowledge of viruses, both plant and animal. From organisms to nucleic acids; morphology of virus particles; the infection process; viruses and other cell components.

Bawden, F. C. 1960. The multiplication of viruses. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 71-116.

Differences between viruses and organisms; the constitution and structure of viruses; analogies with bacteriophage; factors affecting virus multiplication. 103 refs.

Bennett, C. W. 1959. Masked plant viruses. *In* Tunevall, G., ed. Recent progress in microbiology. Symposia held at the VII International Congress for Micro-

biology, Stockholm, 1958. C. C. Thomas, Springfield, Ill. p. 218-223.

Conditions and causes of masking. 11 refs.

Burnet, F. M., and W. M. Stanley, eds. 1959. The viruses. Vol. 2: Plant and bacterial viruses. Academic Press, New York and London. 408 p.

The process of infection and virus synthesis with tobacco mosaic virus and other plant viruses. The biochemistry of plant viruses. Variation and its chemical correlates. Biological cycles of plant viruses in insect vectors. Bacteriophage as a model of host-virus relationship. The initiation of bacteriophage infection. Intracellular multiplication of bacterial viruses. Bacteriophage genetics. Lysogeny. Radiobiology of bacteriophage. Author and subject indexes.

Cochrane, V. W. 1960. Spore germination. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 167-202.

The germination process; maturation and dormancy; spore longevity; spore nutrition and metabolism; germination and temperature, water, pH, light; effects of oxygen and carbon dioxide on germination; some effects of the biological environment. 197 refs.

Gudauskas, R. T. 1960. Plant viral infections as influenced by extracts from some known or suspected hosts of stone-fruit viruses. Doctoral dissertation, University of Illinois. DA 21. 72 p.

Hamilton, R. I. 1960. Studies on the properties of brome mosaic virus and its related antigens. Doctoral dissertation, University of Nebraska. DA 21. 93 p.

Hanson [Hansen], H. P. 1960. Simple designations of potato-infecting and other viruses in accordance with the periodical system of plant and animal virus interrelationships. *Am. Potato J.* 37:187-202.

Identification of individual viruses by their names; natural groupings at the generic level without preference for any particular basis of classification; the proposed nomenclature system; definition of a virus, the basis of the natural virus classification; the principle of the natural periodical system of viruses; comparative survey of typical properties and behavior of plant viruses from the 4 main groups (orders). 47 refs.

Harrison, B. D. 1959. The multiplication of viruses in plants. *Symposium Soc. Gen. Microbiol.* 9:34-60.

Infection of cells by inoculation; the sequence of events in inoculated leaves; virus synthesis in systemically infected leaves; production of variants. 72 refs.

Hartigan, D. 1960. The Australian mistletoe. *J. Forestry* 58:211-218.

Host range; harmful effects; biological factors affecting mistletoe growth; life cycle of Australian mistletoe; control. 12 refs.

Hawker, Lilian E. 1960. Reproduction of bacteria, actinomycetes, and fungi. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 117-165. (91 refs.)

Laxman-Nene, Y. 1960. Studies on the mechanism of inhibition of tobacco-mosaic viral infection by enzymes. Doctoral dissertation, University of Illinois. DA 21. 87 p.

Markham, R. 1959. The biochemistry of plant viruses. *In* Burnet, F. M., and W. M. Stanley, eds. The viruses. Vol. 2: Plant and bacterial viruses. Academic Press, New York and London. p. 33-125.

The purification of plant viruses; protein components of plant viruses; the nucleic acids; the tobacco mosaic virus; properties of the virus protein; the tomato bushy stunt virus; the tobacco ringspot virus; the turnip yellow mosaic virus; the wild cucumber mosaic virus; the tobacco necrosis viruses; the alfalfa mosaic virus; broad bean mottle virus; the southern bean mosaic virus; potato virus X; use of nucleic acid analogs in the control of plant viruses. 273 refs.

Matthews, R. E. F. 1960. Virus inactivation *in vitro* and *in vivo*. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 461-506.

Temperature; radiations; ultrasonic vibration; dessication; high pressures; aging; hydrogen ion concentration; inorganic compounds; metabolites and antimetabolites; substances from organisms. 163 refs.

Murant, A. F. 1955-56. Factors affecting the pathogenicity of certain bacteria to potato tubers. Doctoral dissertation, Imperial College of Science and Technology, London University. GBI 6.

Pavgi, M. S. 1960. Morphology, taxonomy and cytology of the corn rust fungi. Doctoral dissertation, University of Wisconsin. DA 21. 174 p.

Pierce, W. R. 1959. Dwarf mistletoe and its effect upon growth of larch and Douglas fir in western Montana. Doctoral dissertation, University of Washington. DA 20. 158 p.

Pirone, T. P. 1960. Purification, properties, and serology of the cauliflower mosaic virus. Doctoral dissertation, University of Wisconsin. DA 21. 69 p.

Scharen, A. L. 1960. Factors affecting the germination of naturally produced oöspores of Aphanomyces euteiches Drechsler. Doctoral dissertation, University of Nebraska. DA 21. 46 p.

Schomberg, J. 1959. Concepto actual de la constitucion quimica y modo de accion de los virus de plantas [Present concept of the chemical constitution and mode of action of plant viruses]. *Agronomía (Lima)* 26:323-341.

Method of penetration; transmission by plant parasites and by insects; method of action and multiplication of the virus; identification of viruses; methods of control of plant viruses. 18 refs.

Shephard, M. C. 1956-57. Studies on the pathogenicity of Rhizoctonia solani. Doctoral dissertation, Imperial College of Science and Technology, London University. GBI 7.

Siegel, A., and S. G. Wildman. 1960. Some aspects of the structure and behavior of tobacco mosaic virus. *Ann. Rev. Plant Physiol.* 11:277-298.

Structure and composition, mutation, and intracellular behavior of TMV. 177 refs.

Smart, G. C., Jr. 1960. Pathogenicity, morphology, and life cycle of certain isolates of Ditylenchus destructor. Doctoral dissertation, University of Wisconsin. DA 21. 51 p.

Southey, J. F., ed. 1959. Plant nematology. Tech. Bull. No. 7. Ministry of Agriculture, Fisheries and Food, London. 175 p.

Based on the collected lectures from a course held at the N.A.A.S. Regional Headquarters, Bristol. The nematoda; genera of plant parasitic nematodes; importance of cyst-forming eelworms in agriculture; research problems with *Heterodera* species; control; laboratory culture and nutrition studies. 311 refs.

HOST PLANTS

Natural Communities

Butin, H. 1960. Die Krankheiten der Weide und deren Erreger [Diseases of willows and their pathogens]. *Mitt. Biol. Bundesanstalt Land- u. Forstwirtsch., Berlin-Dahlem*, 98:3-46.

Bacteria and fungi recorded in Germany; symptoms and injuries; appearance and biology of the pathogen; host susceptibility; control methods. 74 refs.

Kristensen, H. R. 1960. Virussygdomme hos forstplanter [Virus diseases of forest species]. *Dansk Skovforen. Tidsskr.* 45:155-166.

Symptoms, spread, and control of elm mosaic, elm zonate canker, birch dieback, poplar mosaic, mountain ash variegation, maple mosaic, ash witches' broom. 27 refs.

Nordin, V. J. 1959. Forest disease research in foreseeable future. *In* The next one hundred years in forestry. Proceedings of the Forestry Centennial Conference, Oregon State College, February 1959. Oregon State College, Corvallis. p. 23-28.

Forest disease survey; damage appraisal; viruses; control of forest diseases. 19 refs.

Ornamentals

Ark, P. A. 1959. Fungal and bacterial diseases of orchids. *In* Withner, C. L., ed. The orchids; a scientific survey. Ronald Press, New York. p. 419-430.

Diseases of seedlings and adult plants; control measures. 17 refs.

Jensen, D. D. 1959. Virus diseases of orchids. *In* Withner, C. L., ed. The orchids; a scientific survey. Ronald Press, New York. p. 431-458.

Problems in virus research; how viruses are spread; control of virus diseases; summary of orchid virus diseases (table and explanatory text). 33 refs.

Cultivated Crops

European and Mediterranean Plant Protection Organisation. 1960. Report of the International Conference on Virus Diseases of Citrus, Acireale, Sicily, Sept. 15-17, 1959. Paris. 103 p.

Reports of missions and working committees; notes on the situation in Cyprus, Greece, Yugoslavia, Italy, Spain, Portugal, and France (Côte d'Azur). 29 refs.

Köhler, E. 1960. Die Viruskrankheiten der Kartoffel, nach dem gegenwärtigen Stand der Forschung [Virus diseases of potato, on the basis of the current status of research]. *Angew. Botan.* 34:1-27.

A survey of recent discoveries and views on relevant problems, presented in the form of expanded and systematically arranged annotations to the appended bibliography. I. Descriptions and a classification of the viruses. II. Diagnostic problems. III. Pathological physiology. IV. Epidemiology. V. Control. VI. Resistance buildup. Bibliography of 185 titles.

Mulder, D. 1959. Review of thirty years study of tea diseases in Ceylon. *Tea Quart.* 30:113-116.

Major achievements of the Tea Research Institute.

Paton, A. McE. 1955-56. A study of Pseudomonas, with special reference to species pathogenic to stone fruit trees. Doctoral dissertation, Edinburgh University. GBI 6.

Scaramuzzi, F. 1959. Alcuni aspetti dei rapporti fra virosi e disaffinità d'innesto in frutticoltura [Some aspects of the relation between viruses and stock-scion incompatibility in fruit trees]. *Notiz. Malattie Piante* 47-48:75-102.

Aspects of stock-scion incompatibility; research indicating that similar symptoms may be caused by viruses; selection of propagating material. English summary. 71 refs.

Thresh, J. M., and T. W. Tinsley. 1959. The viruses of cacao. West African Cocoa Research Institute Tech. Bull. No. 7. Crown Agents for Oversea Governments and Administrations, 4, Millbank, London S. W. 1. 32 p.

General features of cacao viruses and criteria for their classification; differences between viruses from different localities, between isolates from different trees in the same locality, and between viruses simultaneously infecting particular trees; relationships between viruses from cacao and those affecting wild hosts and between the various isolates of virus from cacao; suggestions for future work. 61 refs.

Individual Species and Varieties

Commonwealth Bureau of Horticulture and Plantation Crops. 1960. Virus diseases of horse radish. Herbage Abstr. Query File 3450. East Malling, Kent, England.

List of 13 references, compiled in response to a request. Copies available as long as the supply lasts.

Greer, J. E. 1956-57. A study of potato tuber rots occurring in Northern Ireland with special reference to those caused by species of Fusarium and Cylindrocarpon. Doctoral dissertation, Queen's University, Belfast. GBI 7.

LaRose, R., pres. 1958. Tobacco virus diseases: 1st General Session, 2nd International Scientific Tobacco Congress, Brussels, 1958. 24, avenue de Cortenberg, Brussels. p. 59-165.

General reports, communications, discussion. Diagnosis, control, research methods. Text in French and English. 165 refs.

Simmonds, N. W. 1959. Bananas. Longmans, Green & Co., London. p. 366-408.

Ch. XIII—Diseases: Panama disease, banana wilt; leaf spot, Sigatoka disease; bunchy top; minor diseases. 81 refs.

HOST-PATHOGEN INTERACTION

Disposition of the Host; Vulnerability

Bhander, D. S. 1956-57. Studies on the influence of certain mineral nutrients and other factors affecting the susceptibility of the tomato and other solanaceous plants to tomato spotted wilt virus. Doctoral dissertation, Wye College, London University. GBI 7.

Dolliver, J. S. 1960. The role of the host plant in population changes of Pratylenchus spp. Doctoral dissertation, Cornell University. DA 21. 125 p.

Nienhaus, F. 1960. Das Wirtsspektrum von Phytophthora cactorum (Leb. et Cohn) Schroet [The host spectrum of Phytophthora cactorum (Leb. et Cohn) Schroet]. *Phytopathol. Z.* 38:31-68.

Historical review; host spectrum; significance of the fungus source; host spectrum as species identification. English summary. 279 refs.

Otani, Y. 1959. Studies on the relation between the principal components of rice plant and its susceptibility to the blast disease, and on the physiological characters of the blast fungus. *J. Fac. Agr., Hokkaido Univ.* 51(1):3-179.

Literature review and report on extensive studies done at the Botanical Institute, Hokkaido University. 129 refs., many in Japanese.

Entry of Pathogen

Dickinson, S. 1960. The mechanical ability to breach the host barriers. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 203-232.

The mechanism of penetration by force; the breaching of the barriers. 78 refs.

Drachovska, M., and K. Sandera. 1959. [Membrane conductivity in phytopathology.] *Sborník Ceskoslov. Akad. Zemědělkych Věd, Rostlinná Vyroba,* 32:365-375.

In Czech; German summary. 17 refs.

Kerling, L. C. P. 1959. Het oppervlak van het levende blad en de phytopatholoog [The surface of the living leaf and plant disease]. *Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent* 24:577-592.

Leaf surface structure as an indicator of disease symptoms and as an aid in disease control. English summary. 34 refs.

Wood, R. K. S. 1960. Chemical ability to breach the host barriers. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 233-272.

Activity of the pathogen at the surface of the plant; penetration of intact surfaces; invasion of undifferentiated and of differentiated cells; penetration of induced barriers. 107 refs.

Disease Establishment and Spread

Chakravarti, B. P. 1960. Pathogenic capabilities of the wheat stem rust Puccinia graminis tritici in certain rust resistant and tolerant varieties. Doctoral dissertation, University of Minnesota. DA 21. 137 p.

Craig, J. 1960. Physiological, chemical, and morphological plant factors in Zea mays L. associated with diplodia stalk-rot reaction. Doctoral dissertation, University of Illinois. DA 21. 73 p.

Derbyshire, D. M. 1955-56. The relation between mineral nutrition and infection with Verticillium albo-atrum. Doctoral dissertation, Wye College, London University. GBI 6.

Ewing, E. E. 1959. Factors for resistance to pre-emergence damping-off in pea (Pisum sativum) incited by Pythium ultimum Trow. Doctoral dissertation, Cornell University. DA 20. 125 p.

Fadda, N. R. 1955-56. Studies on the mode of action of Corynebacterium fascians (Tilford) Dowson on the host plant. Doctoral dissertation, Imperial College of Science and Technology, London University. GBI 6.

Kazmaier, H. E. 1960. Some pathophysiological aspects of premature defoliation associated with rose blackspot. Doctoral dissertation, Ohio State University. DA 21. 58 p.

Kedar, N. 1958. Resistance of the potato to late blight. Doctoral dissertation, Hebrew University.

Field trials with potato varieties resistant to late blight; resistance and enzymatic activity of the host. Abridged text in English and Hebrew. 62 refs.

Kuhn, C. W. 1960. Concentration and specific infectivity changes of alfalfa mosaic virus during systemic infection in tobacco. Doctoral dissertation, Purdue University. DA 21. 69 p.

Ludwig, R. A. 1960. Toxins. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 315-357.

The toxin concept; toxins and root invasion; toxin production by *Alternaria;* diaporthin and chestnut blight; rice blast; concerning toxins and the vascular diseases; ethylene as a fungal toxin; wildfire and angular leaf spot of tobacco; bacterial canker of stone fruits; toxins and the Victoria blight of oats; plant growth regulators; toxins and the obligate parasites. 164 refs.

Mace, M. E. 1960. Naturally occurring quinones in wheat and barley and their toxicity to loose smut fungi. Doctoral dissertation, North Carolina State College. DA 20. 80 p.

Maine, E. C. 1960. Physiological responses in the tobacco plant to pathogenesis by Pseudomonas solanacearum, causal agent of bacterial wilt. Doctoral dissertation, North Carolina State College. DA 21. 131 p.

McKee, R. K. 1955-56. Studies on the toxicity of solanine and related compounds to microorganisms and on the influence of solanine content on the resistance of potato tuber tissues to fungal infection. Doctoral dissertation, Nottingham University. GBI 6.

Oort, A. J. P. 1959. Over de termen primair en secundair ziek in de fytopathologie [On the use of the terms

primary and secondary disease state in plant pathology]. *Tijdschr. Plantenziekten* 65:142-146.

Explanation of the acceptable usage. English summary. 12 refs.

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Pathogens in soil; pathogens and root infection; pathogen-host relationship. 90 refs.

Singh, G. R. 1959. Studies on development and spread of red rot in a sugarcane plant. Doctoral dissertation, Louisiana State University. DA 20. 112 p.

Smith, G. E. 1960. Effect of antecedent and immediate environment on a species of Cephalosporium producing an antibiotic. Doctoral dissertation, Ohio State University. DA 21. 68 p.

Spalding, D. H. 1960. Mechanisms of the stripe pathogen, Cephalosporium gramineum, in killing cultivated wheat. Doctoral dissertation, Washington State University. DA 21. 94 p.

Strider, D. L. 1959. Studies on the physiology of parasitism as related to scab of cucumber incited by Cladosporium cucumerinum. Doctoral dissertation, North Carolina State College. DA 20. 101 p.

Subramanian, C. V. 1959. Physiology of disease in plants. *J. Madras Univ. B.* 29:9-44.

Physiology of infection; necrosis; leaf spots; vascular wilt diseases; hyperplastic diseases; lengthening of stem. 95 refs.

Wildman, S. G. 1959. The process of infection and virus synthesis with tobacco mosaic virus and other plant viruses. *In* Burnet, F. M., and W. M. Stanley, eds. The viruses. Vol. 2: Plant and bacterial viruses. Academic Press, New York and London. p. 1-31.

General features of the host-virus system; behavior of plant viruses in a local lesion host and in a systemic host; steps in plant virus reproduction. 69 refs.

Williams, Barbara J. 1960. Some host-pathogen relationships in the apple scab disease. Doctoral dissertation, University of Wisconsin. DA 21. 103 p.

Wood, R. K. S. 1960. Pectic and cellulolytic enzymes in plant disease. *Ann. Rev. Plant Physiol.* 11:299-322.

Production of pectic enzymes by plant pathogens; action of pectic enzymes on plant tissue; pectic enzymes in different plant diseases; significance of cellulases in different plant diseases. 149 refs.

DISEASE IN POPULATIONS

Inoculum

Daulton, R. A. C. 1960. Soil temperature and soil moisture factors affecting the survival of eggs of the root-knot nematodes Meloidogyne javanica and M. hapla. Doctoral dissertation, North Carolina State College. DA 20. 104 p.

Garrett, S. D. 1960. Inoculum potential. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 23-56.

Mechanism of inoculum potential; importance of the living hosts as an inoculating agent and as a source of inoculum. 53 refs.

Horsfall, J. G., and A. E. Dimond. 1960. Inoculum and the diseased population. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 1-22.

Inoculum potential; the dispersal of pathogens; control measures and inoculum potential. 25 refs.

Ingold, C. T. 1960. Dispersal by air and water—the takeoff. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 137-168.

Violent spore discharge; passive spore liberation; meteorological conditions in relation to spore liberation; periodicity of spore liberation. 46 refs.

Muskett, A. E. 1960. Autonomous dispersal. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 57-96.

Soil; seeds; plants and plant parts. 107 refs.

Nadakavukaren, M. J. 1960. The effect of soil moisture and temperature on survival of Verticillium microsclerotia. Doctoral dissertation, Oregon State College. DA 21. 72 p.

Schrödter, H. 1960. Dispersal by air and water—the flight and landing. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 169-227.

Transportation forces; the flight; concentration in the air; landing; various problems. 88 refs.

Sreeramulu, T. 1956-57. Studies on the dispersal of spores in the air, with special reference to some pathogenic fungi. Doctoral dissertation, Imperial College of Science and Technology, London University. GBI 7.

Epidemic Development

Marland, A. G. 1956. [Status of the problem of short-time prognosis of Phytophthora on potatoes.] *In* Konf. po Voprosam Zashchita Rastenii Sbornik, Trudov po Zashchita Rastenii. Academy of Sciences, Latvian SSR, Riga. p. 197-203. (In Russian, 11 refs.)

Shestiperova, Z. I. 1960. [The role of cereal grasses in the spread of stem rust.] *Vestnik Sel'skokhoz. Nauki* 6:129-131.

In Russian. *Puccinia graminis* of grain, including transmission by Berberis. 12 refs.

Ullrich, J. 1959. Prognose und Warndienst [Forecasting and warning service]. *Mitt. Biol. Bundesanstalt Land- u. Forstwirtsch., Berlin-Dahlem*, 97:149-156.

Basis and potentialities of forecasting the occurrence of plant diseases and injury. 14 refs.

Van der Plank, J. E. 1960. Analysis of epidemics. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 229-289.

Multiplication of infections; amount of inoculum at its source in relation to epidemics; spread of epidemics; epidemics in relation to the abundance and distribution of host plants; host plants. 64 refs.

Waggoner, P. E. 1960. Forecasting epidemics. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 291-312.

Primary inoculum; dispersal of inoculum; transfer of inoculum; trapping of pathogens; infection; the incubation period; integration; the usefulness of forecasting. 58 refs.

Environmental Factors

Govindu, H. C. 1959. Soil microbiology in relation to plant disease. I. Physical and chemical factors that influence soil microflora. *Mysore Agr. J.* 34:163-168.

Temperature; moisture; aeration and soil texture; light; physical and chemical partial sterilization; lime and soil acidity. 13 refs.

Sayed, M. Q. 1960. The effect of nutrition, pH and nematodes on damping-off disease of pea, tomato and cucumber. Doctoral dissertation, Pennsylvania State University. DA 21. 74 p.

Voronkevich, I. V. 1956. [The effect of soil, as a habitat, on the properties of phytopathogenic bacteria.] *Zhur. Obshchei Biol. Akad. Nauk SSSR* 17:302-310. (In Russian, 18 refs.)

Role of Insects

Black, L. M. 1959. Biological cycles of plant viruses in insect vectors. *In* Burnet, F. M., and W. M. Stanley, eds. The viruses. Vol. 2: Plant and bacterial viruses. Academic Press, New York and London. p. 157-185.

Nature of the insects and viruses involved in biological cycles; history of research on the problem; evaluation of kinds of evidence for multiplication and for absence of multiplication; significance of biological cycles. 85 refs.

Broadbent, L. 1960. Dispersal of inoculum by insects and other animals, including man. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 97-135.

Dispersal by man, by other mammals and birds, by small animals other than insects, by insects. 169 refs.

Cornwell, P. B. 1956-57. Investigations into the ecology and behavior of the mealybug vectors of swollen shoot virus disease of cacao in relation to the spread of the disease and the efficiency of control measures. Doctoral dissertation, London University. GBI 7.

Maramorosch, K. 1960. Leafhopper-transmitted viruses. *Protoplasma* 52:457-466.

Recent advances in the field. Virus transmission and multiplication; site of virus muliplication; diseases of vectors; recent work in Japan; proposal for an international virus-vector laboratory. 69 refs.

Maramorosch, K. 1959. Leafhoppers (Cicadellidae) as vectors and reservoirs of phytopathogenic viruses. *In* Omagiu lui Traian Săvulescu cu prilejul împlinirii a 70 de ani [In honor of Traian Savulescu on the occasion of his 70th birthday]. Editura Academiei Republicii Populare Romîne, Bucharest. p. 421-442.

Review of literature; results of extensive investigations at Rockefeller Institute on 5 viruses. Rumanian summary. 92 refs.

Smith, K. M. 1958. Arthropods as vectors and reservoirs of phytopathogenic viruses. *In* Doerr, R., and C. Hallauer. Handbuch der Virusforschung [Handbook of virus research]. Vol. 4. Springer-Verlag, Vienna. p. 143-176.

Types of arthropod vectors and some of the viruses transmitted by them; relationships between virus and vector in the different insect groups; list of insect-transmitted viruses, with their vectors. Bibliography of 120 titles.

DISEASE CATEGORIES

Types

Bywater, J. 1956-57. Studies on organisms causing root and stem diseases of peas. Doctoral dissertation, Birmingham University. GBI 7.

Cheremisinov, N. A. 1959. [The species of pathogenic fungi causing cob and seed diseases of corn, and ways of infection.] *Botan. Zhur. Akad. Nauk SSSR* 44:916-928. (In Russian, 28 refs.)

Chevaugeon, J. 1959. Le problème des pourridiés en Côte d'Ivoire [The problem of root rots in the Ivory Coast]. *Rev. Mycol.* 24:39-58.

The chief agents of root rot of numerous crops and shade trees in humid tropical areas. Root rot of coffee, cacao, and rubber caused by *Fomes lignosus* in the French Ivory Coast: symptoms, biology of the fungus, importance of rotting of forest trees by the fungus, control methods. 19 refs.

Etheridge, D. E. 1956-57. The influence of moisture and other factors on the activity of heartwood fungi in subalpine spruce. Doctoral dissertation, Imperial College of Science and Technology, London University. GBI 7.

Fisher, D. E. 1956-57. Studies on the stem and fruit rot disease of outdoor tomatoes caused by Didymella lycopersici Kleb. Doctoral dissertation, London University. GBI 7.

Herridge, Elizabeth. 1960. Anatomy and pathology of leaf spots on English holly, Ilex aquifolium (Tourn.) L. Doctoral dissertation, Oregon State College. DA 21. 95 p.

Mechanical injury, insects, copper sprays, and fungal invaders as causal agents.

Kostyuk, P. N., and P. M. Shterenberg. 1959. [Root-endophytous fungi of the grapevine.] *Trudy Vsesoiuz. Nauch.-Issledovatel'. Inst. Vinodel. i Vinograd. "Magarach."* 6:173-180. (In Russian, 30 refs.)

Latch, G. C. M. 1960. Stem diseases of sweet clovers. Doctoral dissertation, University of Wisconsin. DA 21. 110 p.

Causal Agents

El-Arosi, H. M. 1956-57. Synergistic relation between Rhizoctonia solani Kühn and Fusarium solani Snyder and Hansen, in causing a potato tuber rot. Doctoral dissertation, Manchester University. GBI 7.

Hampton, R. E. 1960. Factors affecting the stability of mechanically transmitted viruses of stone fruits. Doctoral dissertation, University of Wisconsin. DA 21. 59 p.

Klinkowski, M. 1959. Aktuelle Probleme der pflanzlichen Virusforschung [Current problems of virus research in plants]. *Mitt. Biol. Bundesanstalt Land.- u. Forstwirtsch., Berlin-Dahlem*, 97:10-36.

Covers fruits, vegetables, and ornamental plants. 106 refs.

Lister, R. M. 1959-60. Soil-borne virus diseases. *Sci. Hort.* 14:90-96.

Mode of transmission; investigations with raspberries and strawberries; control measures. 21 refs.

Lownsbery, B. F., and I. J. Thomason. 1959. Progress in nematology related to horticulture. *Proc. Am. Soc. Hort. Sci.* 74:730-746.

Nematodes parasitizing horticultural crops in the continental U.S.; diagnosis of nematode disease and proof of nematode pathogenicity; manner of plant injury by nematodes; progress in nematode control. 145 refs.

Nur, M. A. 1956-57. A study of powdery mildew diseases with particular reference to Leveillula taurica as a parasite on plants in the Sudan. Doctoral dissertation, University College of Khartoum, London University. GBI 7.

Taylor, D. P. 1960. Biology and host-parasite relationships of the spiral nematode, Helicotylenchus microlobus. Doctoral dissertation, University of Minnesota. DA 21. 58 p.

Ward, C. H. 1960. Occurrence, distribution, and populations of plant parasitic nematodes associated with forage crops in New York State. Doctoral dissertation, Cornell University. DA 21. 81 p.

Individual Diseases

Anderson, L. A. 1960. Studies on the effects of the Cronartium rusts on jack pine, and the epidemiology of these fungi. Doctoral dissertation, University of Minnesota. DA 21. 100 p.

Barnes, J. M. 1960. Investigations on stalk rot of corn caused by Gibberella zeae. Doctoral dissertation, Cornell University. DA 21. 49 p.

Bull, R. A., and J. S. Robertson. 1959. The problems of "little leaf" of oil palms—a review. *J. W. African Inst. Oil Palm Research* 2:355-375.

Nomenclature; geographical distribution and economic importance; symptoms of the spear rot—little leaf complex; cause of the disease; effects of age, genetic constitution, and environment on susceptibility. 37 refs.

Canova, A. 1959. Ricerche su la biologia e l'epidemiologia della Cercospora beticola Sacc. I-V [Research on the biology and epidemiology of Cercospora beticola Sacc. I-V]. *Ann. Sper. Agrar. (Rome)* 13:37-82; 157-203; 477-497; 685-776; 855-897.

Report of extensive investigations, and general review. English summary of each section. 162 refs. (Part V.)

Changari, W. 1960. Studies of Alternaria spp. pathogenic on cruciferae. Doctoral dissertation, University of Florida. DA 21. 169 p.

Cox, A. E., and E. C. Large. 1960. Potato blight epidemics throughout the world. Agr. Handbook 174. U.S. Dept. of Agriculture, Washington, D. C. 230 p.

Life history of the fungus on potatoes; status of the disease, and protective measures employed, in the British Isles, Continental Europe, North and South America, Africa, Asia, and Australasia. 322 refs.

Craighead, F. C., and J. C. Nelson. 1960. Oak wilt in Pennsylvania. *J. Forestry* 58:872-881.

Interpretation and evaluation of work, principally control and transmission studies, on oak wilt from 1952 through 1958. 32 refs.

Croxall, H. E. 1959. Club root of brassicae. *N.A.A.S. Quart. Rev.* 45:20-24.

Important factors and their interaction; resistance; chemical control. 11 refs.

Del Rivero, J. M. 1957-58; 1959. La tristeza de los agrios [Tristeza of citrus fruits]. *Bol. Patol. Vegetal y Entomol. Agr.* 23:15-29; 24:125-172.

I. History, etiology, and distribution. II. Origin and propagation. III. Economic importance and rapidity of spread in infected areas. IV. Symptomatology; progress of the disease; races of the virus. V. Transmissibility. VI. Susceptibility of varieties. 111 refs.

Deshpande, K. B. 1956-57. Studies on the physiology and control of Rhizoctonia solani Kühn. Doctoral dissertation, Imperial College of Science and Technology, London University. GBI 7.

De Weille, G. A. 1960. Blister blight (Exobasidium vexans) in tea and its relationship with environmental conditions. *Neth. J. Agr. Sci.* 8:183-210.

The fungus causing blister blight; incidence in

the field; forecasting attacks. Dutch summary. 37 refs.

Govier, D. A. 1955-56. A virus disease of chrysanthemum. Doctoral dissertation, Exeter University. GBI 6.

Hellmers, E. 1959. Pectobacterium carotovorum var. atrosepticum (Van Hall) Dowson, the correct name of the potato black leg pathogen; a historical and critical review. *European Potato J.* 2:251-271.

Chronological survey of the descriptions of potato black leg, soft rot, and the causal bacteria, 1878-1958; correct name of the bacteria. French and German summaries. 58 refs.

Hirst, J. M. 1955-56. The forecasting, phenology, and control of potato blight. Doctoral dissertation, Rothamstead Experimental Station, London University. GBI 6.

Hodges, C. S., Jr. 1959. Studies on black root rot of pine seedlings. Doctoral dissertation, University of Georgia. DA 20. 127 p.

Huguenin, B. 1959. La "rouille américaine" du Mais due au Puccinia polysora Underw. ["American rust" of maize due to P. polysora]. *Rev. Mycol.* 24:290-323.

Survey covering the history and geographical spread, with special reference to Africa; etiology; symptoms and assessment of degree of susceptibility; transmission; economic importance; control, with special reference to breeding for resistance. 73 refs.

Husain, S. M. 1960. Some aspects of the life history, epiphytology, and control of Alternaria porri (Ellis) Ciferri, the cause of purple blotch of sweet Spanish onions. Doctoral dissertation, Cornell University. DA 21. 148 p.

Kailidis, D. S. 1959. Some aspects of the damping-off of black pine, Pinus nigra Arn. Doctoral dissertation, Michigan State University. DA 20. 99 p.

Kaung, Zan. 1955-56. Tuber infection of potato by the late blight organism, Phytophthora infestans. Doctoral dissertation, Imperial College of Science and Technology, London University. GBI 6.

Khan, S. A. 1959. Studies on Pratylenchus zeae (Nematoda, Tylenchida) on sugarcane in Louisiana. Doctoral dissertation, Louisiana State University. DA 20. 56 p.

Kirby, E. J. M. 1956-57. Host-parasite relations in the choke disease of grasses. Doctoral dissertation, Nottingham University. GBI 7.

Kulkarni, N. B. 1959. Blast disease of rice in Bombay State. *Poona Agr. Coll. Mag.* 50:8-29.

Field observations of the disease; control measures; cost of fungicidal treatment. 85 refs.

McKeen, C. D. 1959. Cucumber necrosis virus. *Can J. Botany* 37:913-925.

Literature review. Results of author's studies: symptomatology; systemic behavior of CNV in cucumber; other properties of CNV; similarities and differences between CNV and TNV. 23 refs.

McMeekin, Dorothy. 1959. The role of the oöspores of Peronospora parasitica in downy mildew of crucifers. Doctoral dissertation, Cornell University. DA 20. 28 p.

Merek, E. L. 1960. A study of the mummy disease of the cultivated mushroom, Agaricus campestris L. ex Fries. Doctoral dissertation, Pennsylvania State University. DA 21. 64 p.

Moriondo, F. 1959. Sul disseccamento degli aghi di Pino cembro in Alto Adige [Needle blight of Pinus cembra in the Upper Adige valley]. *Ann. Accad. Ital. Sci. For.* 8:43-57.

Description of a disease caused by *Phacidium pinicembrae*. 30 refs.

Murphy, H. C., *et al.* 1959. The epidemic of barley yellow dwarf on oats in 1959. Plant Disease Reptr. Suppl. 262. p. 316-384.

Twenty-seven papers reporting aspects of the epidemic in different localities in the United States and Canada: reaction of different varieties of oats; vectors and methods of transmission apparently involved; control methods employed. 59 refs. Yellow dwarf bibliography of 142 titles.

Pawsey, R. G. 1956-57. An investigation in Keithia disease of Thuja plicata. Doctoral dissertation, Nottingham University. GBI 7.

Raymer, W. B., and J. A. Milbrath. 1960. The identity and host relations of the potato late-breaking virus. *Phytopathology* 50:312-319.

Investigation results interpreted in the light of the literature. 43 refs.

Renaud, R. 1959. Oidium du tabac [Oidium of tobacco]. *Rev. Intern. Tabacs* 34:151-160; 176-177; 179-180.

Review of the available information on the morphology, hosts, symptoms, etiology, prophylaxis by regional adjustment of sanitary and cultural practices, and control. 79 refs.

Renaud, R. 1960. La maladie de la feuille blanche (hoja blanca), une nouvelle virose du riz [The white-leaf disease (hoja blanca), a new virus of rice]. *Riz et Rizicult.* 6:31-37.

History and geographic distribution; symptoms; developmental factors; transmission; host plants; resistant varieties and the fight against the insect vectors. English and Spanish summaries. 44 refs.

Scurti, J. C. 1959. La Fusariosi dei gladioli [The Fusaria of gladioli]. *Notiz. Malattie Piante* 49-50:145-160.

Characteristics of some strains of *Fusarium oxysporum* Sch. F. *gladioli* (Massey) Snyd. & Hans. obtained from gladiolus bulbs affected with "yellow disease"; some experimental control methods used by the author; table showing principal methods tested by other workers. English summary. 31 refs.

Thung, T. H. 1960. Hoja blanca, een schadelijke virusziekte bij de rijstcultuur [Hoja blanca (white leaf), a harmful virus disease of rice]. *Landbouwk. Tijdschr.* 72:290-295.

Review of locale, manner of transmission, and similarity to other diseases. English summary. 4 refs.

Van der Zwet, T. 1959. Studies on Phytophthora seedpiece rot of sugarcane and the principal causal organism P. megasperma Drechs. Doctoral dissertation, Louisiana State University. DA 20. 129 p.

Vovk, A. M. 1956. [Cucumber virus 1, paths of its spread in nature, and control measures.] *Trudy Inst. Genet., Akad. Nauk SSSR* 23:296-310. (In Russian, 35 refs.)

Waddell, H. T. 1959. Parasitism of Septoria obesa Syd. and S. chrysanthemella Sacc. on the cultivated chrysanthemum. Doctoral dissertation, University of Florida. DA 20. 90 p.

Walkinshaw, C. H., Jr. 1960. Corky ringspot of potato. Doctoral dissertation, University of Wisconsin. DA 21. 66 p.

Yerkes, D., Jr., and G. Patiño. 1960. The severe bean mosaic virus, a new bean virus from Mexico. *Phytopathology* 50:334-338.

Symptoms; host range; methods of transmission; physical properties. 10 refs.

DISEASE CONTROL

Economic Considerations; General

Chemical Control

Allen, M. W. 1960. Nematocides. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 603-638.

The problem of proving pathogenicity; what a nematocide is; historical review; effect of nematode control on the crop; foliar and seed-borne nematodes; soil-borne nematodes; methods of applying soil nematocides; methods of confining soil fumigants; treatment of living plants (postplanting); physical factors influencing the efficiency of nema- and seed. 79 refs.

Burchfield, H. P. 1960. Performance of fungicides on plants and in soil—physical, chemical, and biological considerations. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 477-520.

Protection of plant surfaces; treatment of soils and seed. 79 refs.

Dransfield, M. 1955-56. The effects of tetrachloronitrobenzene on the soil microflora. Doctoral dissertation, Nottingham University. GBI 6.

Estienne, V., and G. L. Hennebert. 1959. Perspectives des composes organiques de l'etain en phytopharmacie [Outlook of organic compounds of tin in phytopharmacy]. *Agricultura (Louvain)* 7:483-494.

Survey of available information on the status of tin compounds in relation to requirements for a good fungicide. Dutch and English summaries. 26 refs.

Grover, R. K. 1960. Laboratory and field evaluations of antibiotics and synthetic organic fungicides used for the control of Sclerotinia fructicola (Wint.) Rehm. and Sclerotinia laxa Ader. and Ruhl. on cherries. Doctoral dissertation, University of Wisconsin. DA 21. 115 p.

Kreutzer, W. A. 1960. Soil treatment. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 431-476.

Basic concepts; practical aspects. 166 refs.

Lambe, R. C. 1960. The influence of temperature, moisture, and method of application on the fungitoxicity of Mylone and Vapam. Doctoral dissertation, Oregon State College. DA 21. 83 p.

Mann, S. S. 1955-56. The effect of some organic fungicides on organisms responsible for storage rots of the potato. Doctoral dissertation, Edinburgh University. GBI 6.

McCallan, S. E. A., and L. P. Miller. 1958. Innate toxicity of fungicides. *Advances in Pest Control Research* 2:107-134.

Inorganic toxicants; organic toxicants; permeability of spores and distribution of toxicants within the spores; comparison of efficacy of fungicides and other biocides. 81 refs.

McNew, G. L. 1960. Fungicides and bactericides for controlling plant diseases. *In* Reitz, L. P., ed. Biological and chemical control of plant and animal pests. Publ. 61. American Association for the Advancement of Science, Washington. p. 49-71.

Membranes of fungus spores and their penetration; reaction with cell metabolites; detoxification mechanism; stability of fungicides; use of antibiotics. 74 refs.

Miller, P. R., and Jessie I. Wood. 1960. Aircraft in plant disease control. *Agr. Chem.* 15(6):62-63, 113-115.

History and development; techniques employed; scope of utility. 30 refs.

Rich, S. 1960. Fungicidal chemistry. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 553-602.

Destructive properties of fungitoxicants; inorganic and organic fungitoxicants; fashioning fungicides. 128 refs.

Sisler, H. D., and C. E. Cox. 1960. Physiology of fungitoxicity. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 507-552.

Mobilization; uptake; surface of the cell in relation to toxicity; damage to physical structure and organization; interference with intracellular metabolism; biological modification of fungicides; acquired resistance to fungicides; mutation, mitotic disturbances, and morphological aberration; spores *vs.* mycelium. 168 refs.

Spencer, D. M. 1956-57. Studies on direct and systemic fungicidal action. Doctoral dissertation, Wye College, London University. GBI 7.

Van Alphen, Th. G. 1957. Bespuiting met koper op verschillende gewassen. Spraying crops with copper. Centrum Landbouwdocument. Literatuuroverzicht 20. 65 p.

The role of copper in the plant; Netherlands research with reference to agriculture and horticulture; foreign research with reference to agriculture and horticulture. Extensive English summary. 368 refs.

Wain, R. L. 1959. Some chemical aspects of plant disease control. *Roy. Inst. Chem. (London), Lectures, Monographs, Repts.* No. 3:1-21.

Review of investigations on the protective action of a variety of fungicides. 137 refs.

Zummo, Natale. 1960. Studies on chemical control of petal blight of camellia incited by Sclerotinia camelliae. Doctoral dissertation, Louisiana State University. DA 21. 79 p.

Disease-Resistant Varieties

Anderegg, D. E. 1959. Physiological variation among selected isolates of the fungus Diaporthe phaseolorum var. sojae in relation to pathology. Doctoral dissertation, Ohio State University. DA 20. 43 p.

Bordewick, B. E. 1960. Studies on maintenance of virulence of Corynebacterium insidiosum (McCull.) H. L. Jens. in culture and the inheritance of resistance to C. insidiosum in diploid Medicago falcata L. Doctoral dissertation, Purdue University. DA 21. 68 p.

Buxton, E. W. 1960. Heterokaryosis, saltation, and adaptation. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 2: The pathogen. Academic Press, New York and London. p. 359-405.

Physiologic races; heterokaryosis as a factor in variability; saltation as a cause of variability; adaptation as a mechanism of change in plant pathogens. 103 refs.

Day, P. R. 1960. Variation in phytopathogenic fungi. *Ann. Rev. Microbiol.* 14:1-16.

Emphasizes the genetic approach to plant pathology. 143 refs.

Dorst, J. C. 1960. Sources of resistance. *Euphytica* 9:265-271.

History and development of breeding for resistance to abiotic and biotic factors. Dutch summary. 5 refs.

Dutta, S. K. 1960. Pathology of natural races and induced biochemical mutants of Colletotrichum lagenarium Ell. and Halst. and the inheritance of resistance in cucurbits. Doctoral dissertation, Kansas State University. DA 20. 70 p.

Gorter, G. J. M. A. 1959. Breeding maize for resistance to streak. *Euphytica* 8:234-240.

History; recent advances; new sources of resistance. 14 refs.

Green, C. H. 1959. Effects of ploidy differences on horticultural characteristics and disease resistance in watermelons. Doctoral dissertation, Purdue University. DA 20. 118 p.

Hickey, K. D. 1960. The sooty blotch and fly speck diseases of apple with emphasis on variation within Gloeodes pomigena (Schw.) Colby. Doctoral dissertation, Pennsylvania State University. DA 21. 135 p.

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Ramana Rao, B. V. 1960. Radiation-induced plant mutations. A review. *Madras Agr. J.* 47:118-132.

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Reitz, L. P., ed. 1960. Biological and chemical control of plant and animal pests. Publ. 61. American Association for the Advancement of Science, Washington. 273 p.

Symposium presented by Section O, on Agriculture, at the Indianapolis meeting, December 28-30, 1957. Nineteen papers under 3 general headings: I. The public's stake in pest control. II. Recent advances in chemical control. III. Biological control of pests. 588 refs.

Richards, H. R. 1960. Inheritance and expression of loose smut resistance in wheat. Doctoral dissertation, Purdue University. DA 21. 57 p.

Rodriguez, A. E. 1960. Physiological specialization, morphological, and cultural variation in isolates of Helminthosporium turcicum. Doctoral dissertation, Purdue University. DA 21. 72 p.

Seaman, W. L. 1960. Variability in pathogenicity among isolates of Plasmodiophora brassicae. Doctoral dissertation, University of Wisconsin. DA 21. 69 p.

Sechler, D. T. 1960. A study of some plant characters associated with resistance to root lodging in oats. Doctoral dissertation, University of Missouri. DA 21. 117 p.

Shay, J. R. 1960. Breeding vegetable and fruit crops for resistance to disease. *In* Reitz, L. P., ed. Biological and chemical control of plant and animal pests. Publ. 61. American Association for the Advancement of Science, Washington. p. 229-244.

Monogenic versus polygenic inheritance; genetics of pathogens; table of classification of representative vegetable and fruit disease resistance cases according to causal agent, mode of inheritance, and field experience. 46 refs.

Stakman, E. C., and J. J. Christensen. 1960. The problem of breeding resistant varieties. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 567-624.

Importance of disease-resistant varieties; history of attempts to produce resistant varieties; present status; future prospects. 67 refs.

Stanford, E. H. 1960. Breeding field crops for resistance to disease. *In* Reitz, L. P., ed. Biological and chemical control of plant and animal pests. Publ. 61. American Association for the Advancement of Science, Washington. p. 217-228.

Results from plant breeding; shifting pathogens; the meaning of disease resistance. 31 refs.

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Toxopeus, H. J. 1959. Problems involved in breeding for resistance. *Euphytica* 8:223-231.

Review of methods and difficulties. Dutch summary. 11 refs.

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Quarantines

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Gram, E. 1960. Quarantines. *In* Horsfall, J. G., and A. E. Dimond, eds. Plant pathology. Vol. 3: The diseased population. Academic Press, New York and London. p. 313-356.

Natural spread and man-provoked dispersal; legal aspects; evaluation of the quarantine system. 44 refs.

Cultural Control

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General considerations; elements of cultural control; intrinsic measures directly affecting the individuals comprising the host population; extrinsic measures indirectly affecting the individuals comprising the host population; measures affecting elements other than the host population. 131 refs.

Special Problems

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Soil microorganisms and antibiotics. In Armenian. Russian summary. 6 refs.

Almandil, A. R. 1960. Chemical control of spoilage of dates. Doctoral dissertation, Utah State University. DA 21. 74 p.

Bellamy, W. D. 1959. Preservation of foods and drugs by ionizing radiations. *Advances in Appl. Microbiol.* 1:49-73.

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Brian, P. W. 1960. Griseofulvin. *Brit. Mycol. Soc. Trans.* 43, Pt. I: 1-13.

Presidential address. Discovery and production; antifungal activity; mode of action; biological activity and molecular structure; systemic distribution in plants; as an agricultural fungicide. 57 refs.

Briggs, J. D. 1960. Pathogens for the control of pests. *In* Reitz, L. P., ed. Biological and chemical control of plant and animal pests. Publ. 61. American Association for the Advancement of Science, Washington. p. 137-148.

Nematodes; protozoa; viruses; fungi; bacteria. 41 refs.

Brunner, R. 1959. Antibiotica in Pflanzenbau und Pflanzenschutz [Antibiotics in crop production and plant protection]. *Bodenkultur* 10:293-305.

Substances with antibiotic effect from higher plants; treatment of plant diseases with antibiotics; practical application and methods of treatment; results of treatment. 40 refs.

Cook, H. T. 1961. Plant disease control without chemicals. *J. Wash. Acad. Sci.* 51:13-16.

Address of the retiring president of the Botanical Society of Washington, December 6, 1960.

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Hyperparasitism; bacteriophage; predators and antagonistic animals of pathogens; interaction in the fungal and bacterial diseases; complexes associated with nematodes; interferences in virus disease. 167 refs.

Fields, M. L. 1959. The effect of cathode rays on food spoilage fungi. Doctoral dissertation, Purdue University. DA 20. 176 p.

Goodman, R. N. 1959. The influence of antibiotics on plants and plant disease control. *In* Goldberg, H. S., ed. Antibiotics; their chemistry and non-medical uses. D. van Nostrand Co., Princeton, N. J. p. 322-448.

Methods of application; stability and persistence; compatibility of antibiotics with other agricultural chemicals; antibiotic bioassays; additives to antibiotic formulations; phytotoxicity; systemicity; mode of action; antiviral activity; species interactions due to antibiotic exposures; development of bacterial resistance to antibiotics. Annotated bibliography of 376 titles.

Klisiewicz, J. M. 1960. Studies on control of black rot of crucifers with antibiotics. Doctoral dissertation, University of Wisconsin. DA 21. 94 p.

Lagiere, R., and M. Cognee. 1960. Les traitements fongicides et bactericides des semences du cotonnier en Republique Centrafricaine [Fungicidal and bactericidal treatments of cotton seeds in Central African Republic]. *Coton et Fibres Trop.* 15:41-67.

Literature review, 1954-59, showing results of tests conducted at the Institut de Recherches du Coton et des Textiles Exotiques at Bambari. Methods of treatment are compared and recommendations made as to the use of various compounds. English summary. 21 refs.

Lhoste, J. 1960. La protection des cultures par l'enrobage des semences [The protection of plantings by coating of seeds]. *Chim. & Ind. (Paris)* 84:557-570.

Repellants, fungicides, and insecticides used for seed coating; technique of treatment; principal applications of the method; toxic hazards during treatment. English, German, and Spanish summaries. 74 refs.

Mitchell, J. W., B. C. Smale, and R. L. Metcalf. 1960. Absorption and translocation of regulators and compounds used to control plant diseases and insects. *Advances in Pest Control Research* 3:359-436.

Anatomy of leaves, stems, roots, and seeds related to absorption and translocation; absorption and translocation of plant regulators, therapeutants, and insecticides; some advances and common problems. 250 refs.

Moss, V. G., H. J. Viche, and W. Klomparens. 1960. Antibiotic treatment of western white pine infected with blister rust. *J. Forestry* 58:691-695.

Northern Rocky Mountain conditions; cycloheximide investigations; basal stem application; the antibiotic control program. 16 refs.

Pramer, D. 1959. The status of antibiotics in plant disease control. *Advances in Appl. Microbiol.* 1:75-85.

Selective toxicity; absorption and translocation; mode of action. 104 refs.

Sijpesteijn, A. K. 1960. Nieuwe ontwikkelingen op het gebied der systemische bestrijding van schimmelziekten bij planten [New developments in the area of systemic control of mildew diseases of plants]. *Vakblad Biol.* 40:21-30. (27 refs.)

Snyder, W. C. 1960. Antagonism as a plant disease control principle. *In* Reitz, L. P., ed. Biological and chemical control of plant and animal pests. Publ. 61. American Association for the Advancement of Science, Washington. p. 127-136. (13 refs.)

Tarr, S. A. J. 1959. Seed treatment of cotton against diseases and insect pests. *Outlook on Agr.* 2:168-184.

Bacterial blight; problems of seed treatment; treatment with bactericides; methods of application; seed-borne diseases other than bacterial blight. 32 refs.

Turner, J. N. 1959. Control of fungal diseases of fruit in storage. *Outlook on Agr.* 2:229-237.

Types of spoilage; methods of preserving fresh fruit, and their effectiveness. 26 refs.

Upitis, V. V. 1956. [Importance of soil saprophytic fungi in the control of the causative agents of diseases of agricultural plants.] *In* Konf. po Voprosam Zashch. Rast. Sborn., Trudov po Zashch. Rast. Academy of Sciences, Latvian SSR, Riga. p. 181-190. (In Russian, 28 refs.)

Ustinov, A. A. 1956. [Biological principles of measures for controlling plant nematodes.] *Zool. Zhur., Akad. Nauk SSSR* 35:162-172. (In Russian, 15 refs.)

White, L. S. 1959. The incidence and enzymatic activity of molds found in Indiana and Ohio tomatoes. Doctoral dissertation, Michigan State University. DA 20. 142 p.

INSTRUMENTATION; RESEARCH TECHNIQUES

Harris, R. V. 1960. The role of relation in applied biology. *Ann. Appl. Biol.* 48:453-470.

Presidential address, Annual General Meeting of the Association of Applied Biologists, April 1960. An analysis, after 36 years as plant pathologist, of the vital role of positive creative relations in all original scientific practice; suggested procedures for developing an attitude of creative relationship in young scientists. 14 refs.

Johnson, R. M. 1960. Evaluation of five rapid methods for determining smut content of wheat. Doctoral dissertation, University of Maryland. DA 21. 93 p.

Stapp, C. 1959. Die Bedeutung bakteriologischer und serologischer Forschungen für den Pflanzenschutz [The importance of bacteriological and serological research for plant protection]. *Mitt. Biol. Bundesanstalt Land- u. Forstwirtsch., Berlin-Dahlem*, 97: 1-9.

Presidential address, 32nd German Plant Protection Session of the Biologischen Bundesanstalt für Land- und Forstwirtschaft, Hannover, October 6-10, 1958.

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DANSK SKOVFORENing TIDSSKRift 99.8 D23
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TRUDY VSESOIUZnogo NAUCHno-ISSLEDOVATEL'skogo INSTituta VINODELiia I VINOGRADarstva "MAGARACH" 390.9 V96

NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

The first of the monographs arranged by the Committee on Monographs and Reviews is being published by the Society. This is "Barley Yellow Dwarf" by G. W. Bruehl. As a comprehensive treatment of this recently important disease, it is as up-to-date as printing schedules permit. Symptoms (in color) and vectors are illustrated. The volume will be mailed free of charge to members of the American Phytopathological Society. Others, and libraries wishing copies may purchase them for $2.00 from Dr. A. W. Dimock, Business Manager, The American Phytopathological Society, R. D. 2, Ithaca, New York.

Forest pathologist John S. Boyce, Jr., joined the University of Georgia Plant Pathology and Plant Breeding Department July 1 as Professor of Plant Pathology, where he will teach and conduct research on forest tree diseases. Dr. Boyce was with the U. S. Forest Service Southeastern Experiment Station in Asheville, North Carolina, for 11 years before joining the University of Georgia staff.

Phytopathologist A. A. Bell, who recently received his Ph.D. degree from the University of Nebraska, has joined the Botany Department, University of Maryland, College Park. Dr. Bell will teach plant pathology and will continue studies on physiology of spore germination.

A section on plant diseases was prepared by A. J. Riker for inclusion in the 1961 edition of the Encyclopedia Britannica. The ten-page discourse includes a full page of colored illustrations.

Plant pathologist Norman E. McGlohon, a recent graduate of North Carolina State College, has accepted a position as extension plant pathologist in the Agricultural Extension Service, Auburn University, Auburn, Alabama.

Phytopathologist Harry E. Wheeler of the Louisiana State University Department of Botany and Plant Pathology was awarded a National Institutes of Health grant of $59,225 for a 5-year period, beginning June 1, 1961. The grant will sponsor studies on toxins in relation to plant diseases.

The Pacific Division of the Society held its 43rd annual meeting in conjunction with the Pacific Division of the A.A.A.S. June 21-23, 1961, on the Davis campus of the University of California. One hundred and eighty-eight persons registered and 62 research papers were given. An invitational symposium, "Atmospheric Pollutants as Phytotoxic Agents," was presented in cooperation with the American Society of Horticultural Science. Those participating were: M. D. Thomas, E. R. Stephens, O. C. Taylor, H. H. Wiebe, C. G. Shaw, R. F. Brewer, and E. F. Darley, Chairman. Officers elected for 1961-62 were: President, W. A. Kreutzer; Vice President, J. A. Milbrath; and Councilor, G. W. Cochran. C. J. Gould continues as Secretary-Treasurer. A resolution to send a collection of reprints from Pacific Division members to the Plant Pathological Society in Chile was approved. J. D. Menzies, Past President, will handle this project. The Pacific Division will meet jointly with the parent society and the A.I.B.S. at Corvallis, Oregon, August 26-30, 1962.

Phytopathologist Curtis R. Jackson recently joined the staff of the University of Georgia Coastal Plain Experiment Station as Department Head. Dr. Jackson was formerly at the University of Florida Gulf Coast Experiment Station, where he investigated diseases of ornamental crops.

The Department of Botany, Bacteriology, and Plant Pathology of the Louisiana State University as of July 1 was renamed the Department of Botany and Plant Pathology. There is now a separate Department of Bacteriology.

Phytopathologist J. H. Graham, U. S. Regional Pasture Research Laboratory, University Park, Pennsylvania, recently completed a four-week course in the study of radioisotope techniques given by the Oak Ridge Institute of Nuclear Studies.

Plant pathologist Luis Sequeria, who for the past eight years has been associated with the United Fruit Co., joined the staff of the University of Wisconsin as Associate Professor of Plant Pathology on August 1, 1961. For the past year Dr. Sequeria has been on leave of his duties as Scientific Director of the United Fruit Company's Coto Research Station in Costa Rica and has been engaged in research at North Carolina State College.

Phytopathologist W. Wyatt Osborne, who for the past two years has been pursuing graduate work at Rutgers University, has returned to Virginia Polytechnic Institute, Blacksburg, where he will resume his duties as associate extension plant pathologist. Mr. Osborne's graduate work was chiefly in nematology.

After 41 years of service, James G. Dickson retired June 30 from the Department of Plant Pathology, University of Wisconsin. Dr. Dickson is a former President of the Society and of the American Institute of Biological Sciences. He is widely known for his investigations on diseases of field crops, physiology of disease development and disease resistance. Dr. Dickson is presently on a world tour in an advisory capacity.

Phytopathologist J. W. Gerdemann has been granted a six months sabbatical leave effective August 1. He and his family will be traveling in western Europe where Dr. Gerdemann will visit laboratories conducting research on mycorrhizal fungi. He will spend five months in the Department of Botany, Queens College, University of St. Andrews, Dundee, Scotland, where he will be working with Dr. T. H. Nicholson. Dr. Gerdemann received a National Science Foundation travel grant to help defray expenses.

Plant pathologist Richard P. White, for 23 years executive vice president of the American Association of Nurserymen, announced his retirement at the nurserymen's 86th annual convention. Dr. White will be succeeded November 10 by Curtis Porterfield who has been secretary of the national group.

The Shade Tree Laboratories, Department of Entomology and Plant Pathology, University of Massachusetts report the following staff changes: Henry W. Gilbertson, Jr., resigned to accept a position with the Davey Tree Expert Co., Kent, Ohio; Robert S. Inman, who recently completed graduate training in the Department of Plant Pathology, University of Nebraska, joined the staff at the Waltham Field Station, May 7; Francis W. Holmes, on the staff at Amherst, was promoted to associate professor.

Phytopathologist Richard E. Ford, a recent recipient of the Ph.D. degree in plant pathology at Cornell University, has accepted a position at Oregon State College, where he will be working in the Vegetables and Ornamentals Research Branch of the U. S. Department of Agriculture. He will be responsible for virus investigations on peas.

Deaths. Life member Joseph H. Faull, Emeritus Professor of forest pathology at Harvard University, died June 30 at the age of 91. Dr. Faull was a member of many scientific societies and held honorary membership in the Royal Society of Canada.